7 April 2006 | $10

Science

AAAS

Volume 312, Issue 5770

COVER

An ant of genus *Ectatomma* foraging at the Project Amazonas field station in Peru at sunset. An analysis of molecular data and fossils indicates that most subfamilies of extant ants originated 75 to 120 million years ago and diversified by about 60 million years ago. See page 101.

Photo: Corrie S. Moreau

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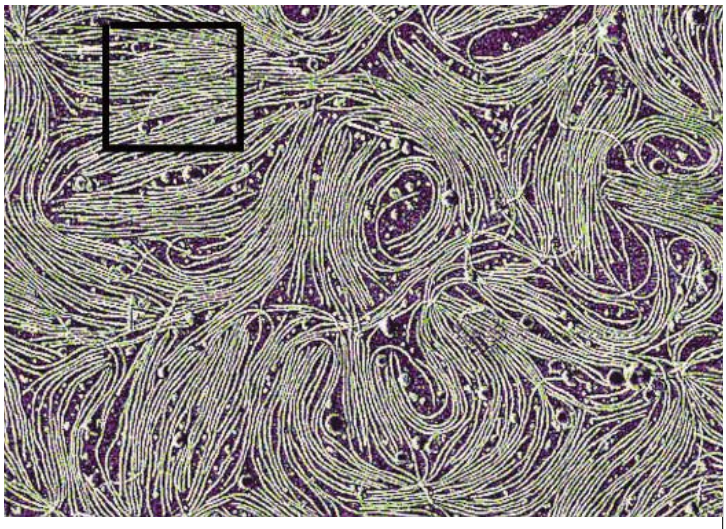

NAM *et al.*

SCIENCE EXPRESS

www.sciencexpress.org

MATERIALS SCIENCE

Virus-Enabled Synthesis and Assembly of Nanowires for Lithium Ion Battery Electrodes
K. T. Nam et al.
Viruses provide a template for growing cobalt oxide nanowires that can be used as battery electrodes, and cobalt oxide–gold hybrid wires that enhance the capacity of nanobatteries.
10.1126/science.1122716

STRUCTURAL BIOLOGY

Recognition of Histone H3 Lysine-4 Methylation by the Double Tudor Domain of JMJD2A
Y. Huang, J. Fang, M. T. Bedford, Y. Zhang, R.-M. Xu
Tandem domains form an interdigitated structure that is required to recognize and demethylate methylated histone tails, a reaction important for gene regulation.
10.1126/science.1125162

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10.1126/science.1126074

PLANT SCIENCE

PIN Proteins Perform a Rate-Limiting Function in Cellular Auxin Efflux
J. Petrášek et al.
Inserting a specific plant protein and its regulated hormone auxin into nonplant cells shows that the protein can move auxin out of cells on its own.
>> Science *Express Brevia by J. Wiśniewska* et al.
10.1126/science.1123542

PLANT SCIENCE

BREVIA: Polar PIN Localization Directs Auxin Flow in Plants
J. Wiśniewska et al.
The polarity of a specific protein controls the flow direction of the hormone auxin in plants, producing a gradient that guides development.
>> Science *Express Report by J. Petrášek* et al.
10.1126/science.1121356

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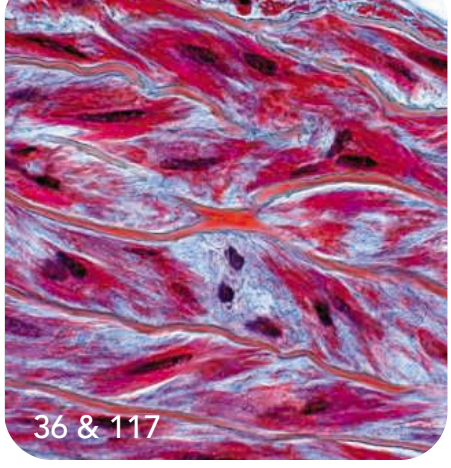

REPORTS *CONTINUED...*

AAAS
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SCIENCE**PODCAST**

Listen to the 7 April edition of the *Science* Podcast to hear about a potential new therapy for Marfan syndrome, insights into the roots of human cooperation, efforts by journals to "clean up the literature" in the wake of recent scientific scandals, and other stories.

SCIENCE**NOW**

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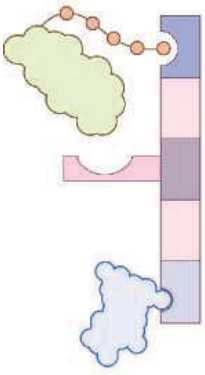
Climate Change on the Flip Side
Falling temperatures are delaying seabird arrival and egg-laying in Antarctica.

Bye Bye Bifocals
Electronic lens that switches focus could someday replace bifocal lenses.

More Telly, More Belly
Study links excess TV exposure to weight gain in preschoolers.



Interacting with the Ub-binding protein p62.

SCIENCE'S **STKE**

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

PERSPECTIVE: What's Ub Chain Linkage Got to Do With It?
I. Kim and H. Rao
Ubiquitin binding proteins may hold the key to substrate fate.

EVENTS
Plan to attend meetings, conferences, or workshops for cell signaling researchers.

Combining pregnancy and science.

SCIENCE **CAREERS**

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

GLOBAL: Pregnancy and the Lab—Feature Index
E. Pain
Next Wave talks to women who have combined pregnancy and scientific work.

US: Two Problems in Search of One Solution
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Can new incentives lure postdocs into the pre-college classroom?

US: Alone in the Lab
J. Kling
Most U.S. institutions lack guidelines for pregnant lab workers, leaving women to identify hazards and solutions on their own.

UK: Managing Your Career Through a Pregnancy
A. Forde
Next Wave investigates the experiences of U.K.-based expectant mothers in the lab.

MISCINET: The Wild World of Doctoral Funding
C. Parks
Doctoral students pay for graduate school in a variety of ways.

GRANTSNET: April 2006 Funding News
J. Fernandez
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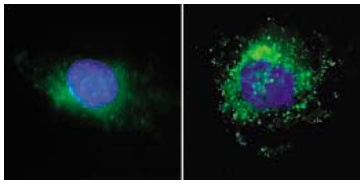

Selective protein recycling keeps cells alive.

SCIENCE'S **SAGE KE**

www.sageke.org SCIENCE OF AGING KNOWLEDGE ENVIRONMENT

PERSPECTIVE: SENS and the Polarization of Aging-Related Research
D. A. Gray and A. Bürkle
Controversy surrounds the "strategies for engineered negligible senescence" concept and conference.

NEWS FOCUS: Tag-Team Recycling
M. Leslie
Mechanisms for protein disposal interact.

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EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Repeating Ring Properties

Two new outer rings and moons were recently discovered around Uranus. Using the infrared Keck adaptive optics system, **de Pater *et al.*** (p. 92) show that the rings are blue and red like Saturn's E and G rings. Blue ring R1 is associated with moon Mab, and Saturn's E ring hosts the active moon Enceladus. This correspondence suggests that Mab may be the source of ring material and the blue color, because only small grains survive gravitational forces, solar radiation pressure, and electromagnetic forces. Ring R2 is as red as Saturn's G ring and shows the same forward- and back-scattered light ratios. Both the uranian and saturnian rings are also at similar locations in planetary radii.

Beginning with C–H Bonds

Carbon-hydrogen bonds in organic molecules and biopolymers are among the least reactive chemical groups, and in chemical synthesis, a C–H bond is first activated by oxygenation or halogenation reactions that can be unselective or difficult to control. **Godula and Sames** (p. 67) review recent progress in transition metal catalysis that has allowed direct, selective formation of carbon-carbon bonds from isolated C–H bonds. These synthetic routes offer great potential for increased synthetic efficiency in preparing complex molecules such as drug precursors.

From Quantum Kittens to Flying Cats

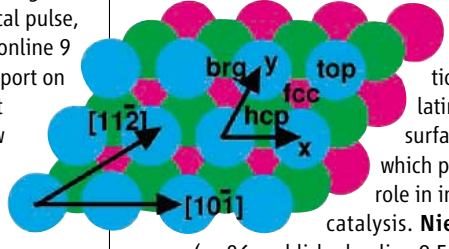
Quantum information processing will require the reliable preparation of quantum states of matter. While these are easy to specify theoretically, experimental realization of such states has been difficult, especially the type of "flying" states that are expected to be useful for quantum communication purposes. By subtracting a single photon from a squeezed coherent optical pulse, **Ourjoumtsev *et al.*** (p. 83, published online 9 March; see the Perspective by **Gisin**) report on the production of small Schrödinger cat states, or Schrödinger kittens, and show that these kittens can be grown into cats through a suitable amplification and distillation process.

Superalloying Cobalt

Superalloys, which are based on iron, cobalt, or most commonly nickel, can be safely used at temperatures in excess of 0.7 of the absolute melting temperature, unlike conventional alloys, which are prone to creep and oxidation. Through the addition of solutes like aluminum or titanium, or both, a two-phase equilibrium microstructure forms that consists of γ and γ' phases; the latter phase is largely responsible for the elevated-temperature strength of the material and its incredible resistance to creep. Cobalt superalloys typically have lower strengths than those based on nickel, which is why the latter has dominated in applications. However, **Sato *et al.*** (p. 90) now show than a ternary cobalt alloy based on the addition of aluminum and tungsten has properties that compete with those of the nickel superalloys.

H_2 Leaves Pt Unexcited

The Born-Oppenheimer (B-O) approximation, which treats nuclear and electronic motion independently during chemical interactions, is a cornerstone of computational modeling. Without it, theoretical analysis of even small molecule reactions in the gas phase would prove dauntingly complex. However, the ease with which electrons can be excited at metal surfaces has cast doubt on the validity of the approximation for simulating molecule-surface collisions, which play a major role in industrial catalysis. **Nieto *et al.*** (p. 86, published online 9 February; see the Perspective by **Wodtke**) show that data for scattering and dissociative adsorption of H_2 at a platinum surface are well predicted with a density functional theory approach with the B-O criteria intact. The absence of Pt electronic excitation during the H_2 interaction suggests that accurate modeling of a wide range of heterogeneous reactions should be feasible.



Hunting Hurricane Causes

A number of different factors can affect the formation and development of hurricanes, including sea surface temperature (SST), lower tropospheric humidity, vertical wind shear, and large-scale atmospheric circulation patterns. Which of these factors are most important and which are responsible for the increase in global hurricane intensity observed since 1970? **Hoyos *et al.*** (p. 94, published online 16 March) use a method based on Bayesian statistics and information theory to isolate the causes of the trend from short-term variability, for all of the major ocean basins where these storms occur. They conclude that only rising tropical SSTs have had a significant influence on the recent multi-decadal trend.

No Pain, No Gain

Societal behavior is complex and multifaceted. One complicated question is the conditions under which we cooperate with others for mutual gain. Experimental results using a public goods game suggest that the threat of costly punishment of free-riders by altruistically minded souls suffices to maintain groupwide compliance. **Gürerk *et al.*** (p. 108; see the Perspective by **Henrich**) show that if allowed to choose freely, individuals first elect to join a sanction-free game where punishment is not permitted. As successive rounds are played, they come to appreciate that cooperation yields greater rewards, so they switch to the sanctioning regime where punishment (which makes free-riding costly) is allowed and themselves become active monitors of compliance.

Continued on page 17

CREDITS (TOP TO BOTTOM): M. SHOWALTER; I. DE PATER, H. HAMMEL, AND S. GIBBARD; NIETO *ET AL.*

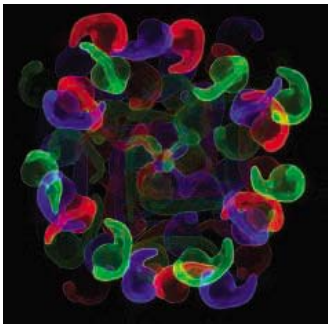
Continued from page 15

Ant Family Tree

Ants are a dominant feature of terrestrial ecosystems and yet we know surprisingly little about their evolutionary history. **Moreau *et al.*** (p. 101; see the cover) sequenced DNA from multiple genes for a representative sample of ant species from around the world to reconstruct an ant family tree. A single group, the *Leptanillinae*, lies at the base of the tree, while all the other groups fall into two major clusters. By using fossils to calibrate the rates of DNA evolution in ants, they conclude that present-day ants arose approximately 140 to 168 million years ago. However, ant diversification only took off ~100 million years ago, immediately after the rise of flowering plants, the angiosperms.

MicroRNAs in Embryogenesis

Early in animal development, the embryo switches from using maternally provided messenger RNA (mRNA) transcripts to expressing mostly zygotic genes. During this maternal-to-zygotic transition, a large number of maternal mRNAs are somehow eliminated. **Giraldez *et al.*** (p. 75, published online 16 February; see the Perspective by **Cohen and Brennecke**) examined possible microRNA (miRNA)-based mechanisms and identified 203 putative targets for the zebrafish miRNA miR-430, which is specifically expressed at the maternal-to-zygotic transition. Hundreds of miR-430 target mRNAs are maternally expressed during early development, and miR-430 can promote their deacetylation and decay. Thus, during the maternal-to-zygotic transition in zebrafish embryogenesis, miR-430 plays a critical role.



CREDIT: ANTONIO J. GIRALDEZ

Serotonin and Liver Regeneration

The liver can regenerate after severe injury or surgery, even when up to 70% of the tissue has been removed. **Lesurtel *et al.*** (p. 104) report that in a mouse model, serotonin carried by platelets circulating in the blood plays a role in the regenerative process. Liver was found to express serotonin receptors. Mice with impaired platelet function had a reduced regenerative response, but when treated with a serotonin receptor agonist, hepatocyte proliferation was restored. Liver regeneration in mice lacking peripheral serotonin was also restored when their platelets were reloaded with serotonin. Therapeutic treatment with serotonin receptor agonists may thus be useful in tissue recovery.

Limits to Evolutionary Flexibility

Genetic mutations are the substrate for evolution. Genes conferring fitness can accumulate multiple mutations during a period of selection. There are, of course, many potential evolutionary trajectories for the appearance of these mutations. However, it is likely that not all trajectories are available because the fitness of individual mutations may depend on the genetic background in which they appear. **Weinreich *et al.*** (p. 111) chart the available evolutionary trajectories for five mutations in β-lactamase in *Escherichia coli*, which together confer a 100,000-fold increased resistance to the antibiotic cefotaxime. Only 18 of a potential 120 routes to high fitness are accessible to selection, due to pleiotropic effects of the mutations on the enzyme.

Therapy for Marfan Syndrome

Marfan syndrome (MFS) is a hereditary disorder characterized by systemwide defects in connective tissue. People with MFS have a greatly increased risk of developing an aortic aneurysm, a bulge in the wall of the aorta that can rupture and cause life-threatening internal bleeding. Studying a mouse model of MFS, **Habashi *et al.*** (p. 117; see the news story by **Travis**) found that aneurysm formation is accompanied by activation of the transforming growth factor–β (TGF-β) signaling pathway in the aortic wall. Treatment of the MFS mice with losartan, a drug recently shown to antagonize TGF-β signaling in other disease states, almost completely normalized the aortic phenotype in the MFS mice, even after an aneurysm had formed. Losartan is already widely used to control high blood pressure, and the authors suggest that a prospective clinical trial in MFS patients is warranted.

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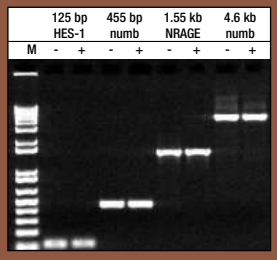

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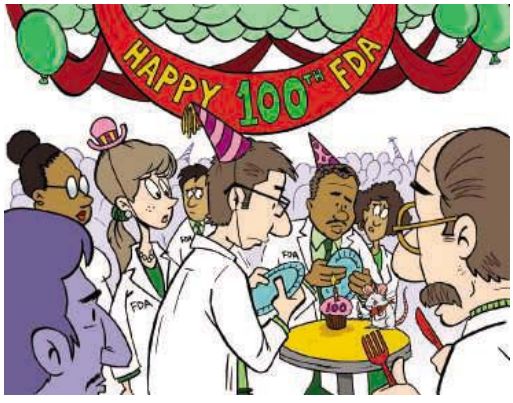
Donald Kennedy is Editor-in-Chief of *Science*.

FDA Centennial

THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) IS TURNING 100. ITS LONGEVITY IS IN MANY ways a political miracle. Originally a chemistry unit in the Department of Agriculture, it was founded soon after Upton Sinclair's scary portrait of turn-of-the-century meat production in *The Jungle*. Several metamorphoses followed over the next half-century: drugs were added; food laws were amended; and the agency moved to the Department of Health, Education and Welfare after World War II. It still bears remnants of that history: the FDA gets its appropriation from Agriculture committees in Congress and its oversight from Health and Commerce committees. What that meant, as I discovered when I became FDA commissioner in 1977, was that you go to rural conservatives for your money and to consumer-friendly urbanites for punishment or occasional praise.

Somehow the FDA has managed to retain a fairly respectable image with U.S. citizens while holding some regulatory responsibility for about 25 cents out of every dollar they spend. Food safety is a serious public concern, and most people like the fact that the FDA protects them from things such as bad seafood and aflatoxin in corn. The approval process for drugs and medical devices is trickier. Industry argues that FDA regulation keeps valuable therapies away from us, whereas consumers claim that it approves too many drugs with harmful side effects. Yet most Americans think the agency is staffed by seasoned professionals who have the public interest at heart and do their jobs with professional skill.



What I'd tell the few old friends left at the FDA is that you deserve better than you're getting. Many of the current problems aren't your fault, beginning with the alarming fact that in the past 6 years, the agency has had a confirmed commissioner for less than 20 months. That's a clear signal that the FDA doesn't matter much to the folks in the White House, and it won't elevate agency morale. Acting FDA Commissioner Andrew von Eschenbach, also Director of the National Cancer Institute (NCI), has now been nominated for the top job, but the Senate may ask why he's been allowed to develop drugs at NCI and then approve them at the FDA. After he explains that, his next challenge will be to deal with the Plan B contraceptive, which remains unavailable despite an advisory committee recommendation and is now hung up pending a long public comment period to evaluate whether it should available over the counter to women over the age of 17.

Those imposed burdens accompany other issues falling into the FDA's responsibilities. Drug safety questions arose over the use of antidepressants by adolescents. Then came Vioxx and other COX-2 inhibitors and concerns about cardiovascular side effects. In the medical devices area, problems surfaced regarding the initial approval and postmarketing safety surveillance of certain pacemakers produced by the Guidant Corporation. Finally, there is the ethical controversy about patient protection in the clinical trial for a blood substitute called PolyHeme. Northfield Laboratories seeks approval for its use in treating hemorrhagic blood loss after trauma. In the trial, one group of patients will get PolyHeme while a control group gets saline along with blood transfusions. How do you get informed consent from a trauma victim? You waive the requirement for it! The Office of Human Research Protections objected vainly to that for over 2 years, and the FDA has been dressed down by a furious Senator Charles Grassley over its prolonged unresponsiveness. The plan is that the trial sites will deliver "community briefings" to help citizens decide whether to be subjects. To opt out, you call the company, request a blue hospital-style bracelet, and then wear it to warn paramedics that you're not part of the experiment! If this is an adequate proxy for informed consent, I am a coloratura soprano.

Back in defense of the FDA, it's not their fault that they have been chronically underfunded. Despite the recent requirement that pediatric drugs be approved and the need to monitor increasingly international drug production, appropriations have not accompanied the new mandates, and earmarks have cut the budget further. The White House seems to have forgotten who's in charge there, and Congress is considering a new statute that lets patients who have run out of treatment options get new drugs that have not been fully tested (remember Laetrile?). It's really too bad that we can't find a few friends in high places for the FDA. After all, it's their birthday; how about a little love? Or maybe money?

– Donald Kennedy

10.1126/science.1127486

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CLIMATE SCIENCE

Lining up the Ducks

Redistributing mass alters rotational speed.

Tide gauge and satellite measurements indicate that global mean sea level has increased by 1.5 to 2.0 mm/year during the 20th century. A significant fraction of this increase is ascribed to glacial melting caused by warming, with the remainder due to thermal expansion of the oceans. Because glacial melting redistributes Earth's mass from high latitudes, where water is stored as ice, to lower latitudes, any appreciable melting should change the planet's rate of rotation, as when a spinning figure skater extends her arms, and the orientation of the rotational vector, which should move as mass shifts. However, the simultaneous agreement in the movement of the rotational pole, the historical observations of ancient eclipses (which allow trends in the length of day to be computed), and space-based gravity measurements (which reflect mass redistributions) has seemingly precluded any major amount of ice melting during the past hundred years.

Mitrovica *et al.* challenge that view with a new theory of rotational stability that involves reformulating how the shape of Earth has responded to glacial melting. In this way, they show how the full suite of Earth rotation and geodetic observations can be reconciled with those of glacial melting and associated sea level rise. — HJS

Earth Planet. Sci. Lett. **243**, 390 (2006).

ECOLOGY/EVOLUTION

The Origin of Natural Selection

The initial stimulus for Darwin's insight into natural selection as the engine of speciation and evolution is often believed to be the radiation of the Galapagos finch. In fact, his thoughts were triggered by the mockingbirds of the endemic genus *Nesomimus*, which exhibit a variety of allopatric forms on the islands of the same archipelago.

Nesomimus parvulus.

Although the finches have been studied intensely by generations of evolutionary biologists, the mockingbirds have suffered a benign neglect. Darwin's view was that the Galapagos mockingbirds, which were recognized as three species on the basis of the *Beagle* specimens (a fourth being added after his death), had descended from a single colonization event perpetuated by wayfarers from Chile or Argentina.

Arbogast *et al.* have tested this view by analyzing mitochondrial DNA sequences. The molecular phylogeny indicates that the Galapagos mockingbirds are indeed monophyletic, but that their closest relatives in the genus *Mimus* are now found in North and Central America, rather than the nearest part of the mainland (Ecuador), and that *Nesomimus* appears to belong within the ancestral genus. Their analysis also illuminates the finer-grained relationships amongst the Galapagos mockingbirds, revealing the wind-aided routes by which they diversified and how their history compares to that of the finches. — AMS

Evolution **60**, 370 (2006).

GEOPHYSICS

Not the Fault of Compaction

Deltas represent a huge accumulation of sediment; large ones are often the sites of major cities, such as New Orleans. Several processes—compaction of the sediment, withdrawal of ground water and oil (which accelerates compaction), and sea level rise—lead to subsidence and inundation of the deltas and to associated problems for their cities. These are compounded when the supply of new sediment is interrupted, as is commonly the case.

On the other hand, subsidence can also be related to faulting induced within the huge sediment pile. By analyzing a large number of leveling benchmarks tied to modern Global Positioning System data, Dokka shows that subsidence over the past 50 years around New Orleans has been dominated by such a fault and not by sediment compaction driven by groundwater pumping as has been presumed. The fault has downthrown a 200-km-wide block extending north of New Orleans out into the Gulf of Mexico. The size of the fault block has made it difficult to recognize in local benchmark surveys, which thus could not reveal the absolute rates. Subsidence attributed to faulting may have reached about 17 mm/year around 1970 and several millimeters per year recently, which is comparable to the presumed compaction rate. — BH

Geology **34**, 281 (2006).

MATERIALS SCIENCE

Crayfish Crystals

A number of studies have shown that biomineralization can occur through the sudden transformation of amorphous calcium carbonate (ACC) into its crystalline forms. In crayfish, for example, the exoskeleton consists primarily of a composite of chitin and protein microfibrils and calcium carbonate. Sugawara *et al.* examine the role of a recently isolated crayfish peptide, known as CAP-1, on the formation of calcium carbonate crystals. Chitin was spun-coated onto glass, where it formed a layer of fibrils and was then covered with a supersaturated solution of calcium carbonate and a small amount of CAP-1. The authors observed the growth of micrometer-sized crystals that were composed of assemblies of nanocrystals and were all found to have the same *c*-axis orientation. The crystals were connected to the chitin through CAP-1 and formed a nanocomposite. Crystallization occurred within the first 5 min after mixing, indicating that a sudden transformation of ACC occurs in the presence of CAP-1. By removing the lone phosphoserine residue of the peptide, the authors observed oriented crystal growth but

Continued on page 23

Continued from page 21

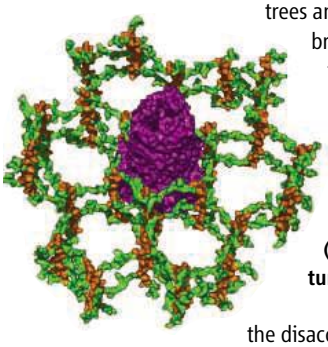
with larger crystals, indicating that the phosphate group may play a role in limiting crystal growth through the stabilization of the ACC. — MSL

Angew. Chem. Int. Ed. **45**, 10.1002/anie.200503800 (2006).

BIOCHEMISTRY

A NAG-NAM Network

Any mention of biological polymers often serves as shorthand for proteins or nucleic acids. Sugar-based macromolecules come to mind less readily even though they constitute some of the most abundant and visible manifestations: cellulose in trees and chitin in invertebrate exoskeletons. A third and equally important member of this group is the disaccharide building block (NAG-NAM) of the bacterial cell wall, whose essential contribution to survival is amply illustrated by the use of lysozyme in the laboratory and penicillin (and its descendants) in the clinic.



An angled view of the peptide (green), glycan (orange), and TolC turret (purple).

Merouen *et al.* describe the NMR structure of a synthesized fragment incorporating two of these disaacharides and their two pendant peptides, which cross-link the glycan strands in vivo. Modeling this fragment into an average-length glycan (10 saccharide units) yields a helix with the pentapeptides emerging at 120° to each other. Factoring in the critical assumption that these strands run perpendicularly to the membrane surface makes it feasible to situate these helices within a honeycomb structure with pores of diameter 70 Å, which snugly accommodate the TolC efflux channel that bridges the periplasm and outer membrane. — GJC

Proc. Natl. Acad. Sci. U.S.A. **103**, 4404 (2006).

DEVELOPMENT

Trading Accuracy for Speed

Genetic damage is potentially very dangerous to cells, so when it does occur, repair usually follows right away. During cell division, DNA replication forks grind to a halt at sites of damage, activating a "checkpoint" that delays cell-cycle progression until repair is complete. But for some developmental processes, cell-cycle timing is itself critical, as in the asynchronous cell divisions that occur in the two-cell *Caenorhabditis elegans* embryo. How do developing nematodes keep to schedule when confronted by a checkpoint?

Holway *et al.* show that during early *C. elegans* embryonic development, checkpoint activation by damaged DNA is prevented by the genes *rad2* and *gei-17* but remains responsive to developmental inputs that regulate timing. *gei-17* suppresses the repair checkpoint by facilitating replication through damaged DNA. Although the normal replication machinery cannot cope with damaged DNA, the so-called translesion DNA polymerase *polh-1* enables the *C. elegans* embryo to overcome genomic damage and avoid a fatal delay in cell division. But this is a tradeoff: Translesion polymerases are error-prone, and embryos opt for survival at the cost of an increase in mutations. — GR

J. Cell Biol. **172**, 999 (2006).

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<< Holding Onto Two Jobs

Proteins in the Bcl-2 family are critical for programmed cell death (apoptosis). In mammals, pro-apoptotic proteins (such as Bax) stimulate mitochondrial fragmentation and the release of cytochrome c; anti-apoptotic proteins (such as Bcl-2 and Bcl-xL) oppose these processes. In the nematode *Caenorhabditis elegans*, CED-9, a protein related to Bcl-2, sequesters the caspase-activating protein CED-4 and thereby inhibits apoptosis. Delivani *et al*. expressed CED-9 in mammalian cells and found that, though localized to mitochondria, it failed to inhibit Bax-induced release of cytochrome c from mitochondria or apoptosis. However, CED-9 promoted remodeling of the mitochondrial network into large perinuclear structures and inhibited the mitochondrial fragmentation associated with apoptosis. EGL-1, which binds to CED-9, promoted mitochondrial fragmentation in mammalian cells (as it does in *C. elegans*) and inhibited CED-9–mediated mitochondrial fusion. When coexpressed in mammalian cells, CED-9 bound to mitofusin, a protein that promotes mitochondrial fusion. Similarly, Bcl-xL bound to mitofusin when cotransfected into mammalian cells and promoted mitochondrial fusion. The authors suggest that Bcl-2 family proteins are involved in regulating mitochondrial fission and fusion in addition to their role in regulating apoptosis. — EMA

Mol. Cell **21**, 761 (2006).

CREDIT: MEROUEH *ET AL., PROC. NATL. ACAD. SCI. U.S.A.* **103**, 4404 (2006)

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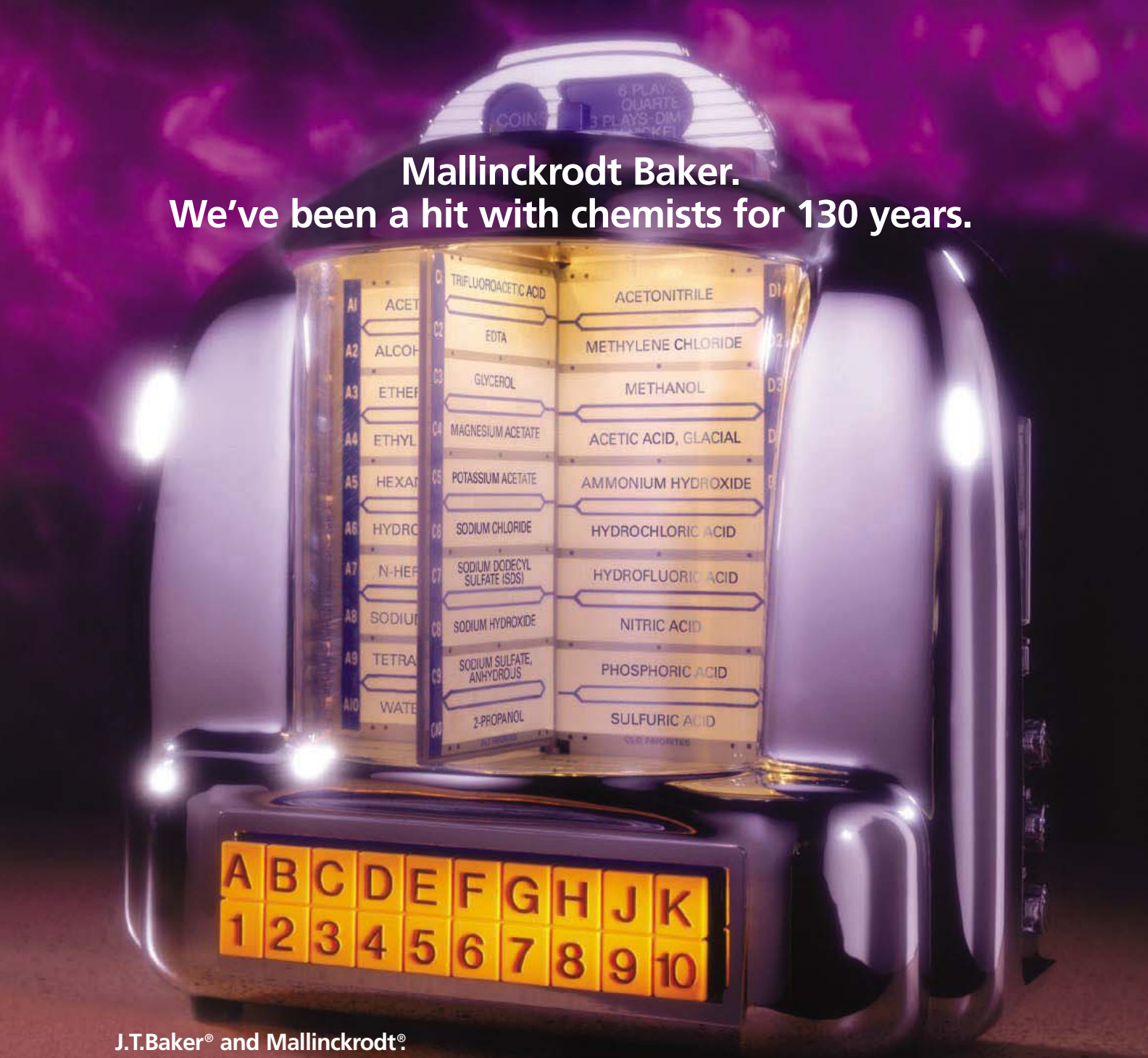

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ITALY'S LIFE SCIENCES SECTOR IS GAINING MOMENTUM

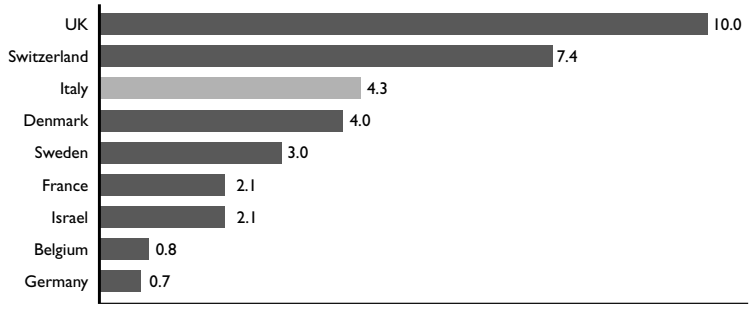
A breeding ground for biotech companies

Italy's Life Sciences industry is becoming ever more appealing for multinational companies seeking to pursue biotechnology and pharmaceutical research. The sector is spurred on by the strong interaction between academia and business environment, a vibrant medical and hospital system, the capacity of world class scientists to produce leading-edge research as well as government support.

Italy's upsurge in the Life Sciences is also proved by a strong performance in the product pipeline with 21 drugs in clinical trials (particularly in Oncology and Neurosciences), which makes it rank ahead of some major European countries like France, Germany and Sweden, if we compare the number of companies with products in pipeline.

Performance Index of Biotech Companies

(Number of products/Number of companies)



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Competitive advantages for international investors

Italy's competitive advantage for international investors is also represented by the skilled workforce. Its R&D professionals – 6,000 researchers employed by businesses, a pool of 20,000 university researchers, 200,000 students and 35,000 graduates annually in Biotechnology, Pharmacy and Medicine – are extremely productive, with creativity second to no competitor country worldwide. As a proof, Italy ranks top in Europe for patent productivity and impact rate of publications.

Start-ups and new business initiatives can count on the support of a network of science parks specialized in life sciences, with a track record of excellence in Biotechnology, Biomedical technology, Diagnostics, Genomics. Besides, labor, business and clinical trials costs are internationally competitive with respect to USA, UK, France and Germany.

Innovation Spotlight

Italy to Launch Europe's First Institute for Regenerative Medicine

Modena – The University of Modena and the Eye Bank Foundation of Venice have joined forces to create a public/private partnership forming the Research Center for Regenerative Medicine. It will become the first such center in Europe focused on stem cell therapy for treating vision disorders caused by tissue/organ damage and genetic defects.

Italy Leads Development of Gene Expression Atlas

Naples – The Telethon Institute of Genetics and Medicine (TIGEM) is spearheading a team made up of 12 major European research institutes to develop the first comprehensive atlas of gene expressions with an estimated identification of 30,000 genes.

Italian and American Researchers Team Up on Heart Stem Cell Breakthrough

Rome – Researchers at La Sapienza University in Rome recently teamed up with John Hopkins University to conduct the first study using stem cells to repair the same type of organ from which they were derived. The promising results were presented at the American Cardiology Congress (ACC).

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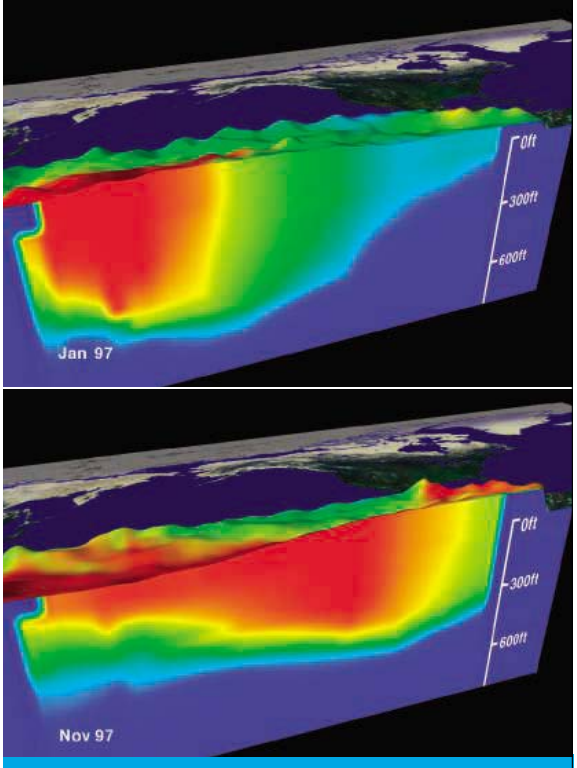

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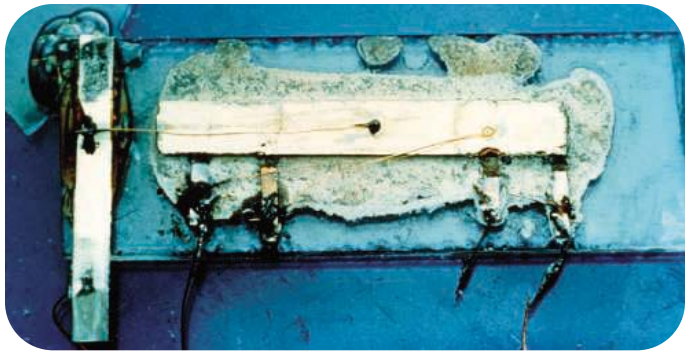
RESOURCES

CLIMATE SWINGS

El Niño and La Niña periodically disrupt wind patterns and ocean temperatures, bringing deluges to some areas of the world and drought to others. Whether you're after background information on the climate phenomena or the latest data on warm-water volume in the tropical Pacific Ocean, zoom over to the El Niño Theme Page from the U.S. National Oceanic and Atmospheric Administration (NOAA).

El Niño occurs when warm water that normally pools in the western Pacific (top) sloshes toward South America (bottom). By contrast, cool water predominates along the equator during La Niña. The Basics section explains these climatic extremes with primers, animations, and other resources. Visitors will also find the latest forecast—we're currently in a La Niña episode that scientists predict will continue for the next 3 to 6 months. Researchers can trawl numerous data sets from NOAA and other sources, which record variables such as atmospheric water vapor and sea level. >> www.pmel.noaa.gov/tao/elnino/nino-home.html



EXHIBITS

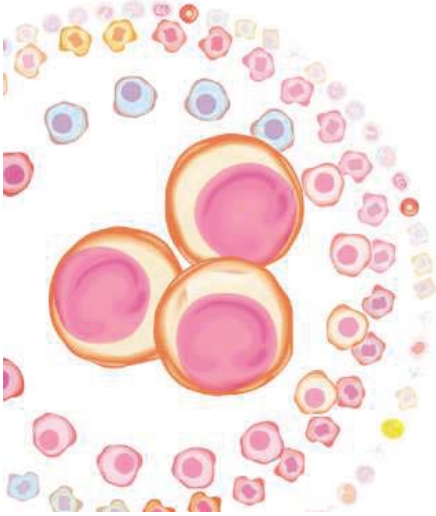
Electrification Project

This centimeter-long lump of solder and wires (above) is one of the original integrated circuits, built by Jack Kilby of Texas Instruments in 1958. To learn more about the history of the shrinking microchip and other developments that electrified our world, plug into the IEEE Virtual Museum from the Institute of Electrical and Electronics Engineers. Subjects of the nine exhibits include Thomas Edison and nanotechnology. A presentation on electronic music resurrects pop songs you tried hard to forget to demonstrate instruments such as the Moog synthesizer. It meshed tone-producing circuits, filters, and other modules so that for the first time musicians could create new sounds. Visitors can divert to minibiographies of figures such as integrated circuit co-inventor Robert Noyce of Fairchild Semiconductor and backgrounders on buckyballs, vacuum tubes, and other technologies. >> www.ieee-virtual-museum.org/index.php

DATABASE

The Crustacean Cure

Ever since Alexander Fleming spied an errant mold growing on a culture dish, most antibiotics have come from fungi and bacteria. A new generation of the drugs could hail from creatures best known as a tasty dish: shrimp, which battle microbial interlopers using peptides known as penaeidins. Immunologists, drug designers, and other researchers can learn more about these molecules at PenBase, sponsored by an international team of scientists. A database lists amino acid and DNA sequences for 28 of the bug-killing compounds, more than half of which are products of the Pacific white shrimp (*Litopenaeus vannamei*). The site also serves up a nomenclature guide, a bibliography, and a roster of PCR primers for duplicating penaeidin genes. >> www.penbase.immunaqua.com



EDUCATION

<< Stem Cell Basics

How does therapeutic cloning differ from reproductive cloning? What distinguishes the stem cells in umbilical cord blood from embryonic stem cells? Students tussling with such questions can find help at a new primer from the University of Michigan, Ann Arbor. The site's centerpiece is a six-part multimedia tutorial that explores topics including the different types of stem cells and their potential applications in drug testing. Visitors will learn, for example, that blood-forming stem cells in the umbilical cord have begun to specialize, so they can't produce the same assortment of tissues as embryonic stem cells can. >> www.lifesciences.umich.edu/index.html

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ANCIENT IRISH STONES REINTERPRETED

Tall stones bearing odd markings, the earliest signs of written Celtic language, have been found in many places in Ireland and Scotland. The Irish for centuries believed these so-called Ogham Stones, named after the language, to be ceremonial objects carved by pre-Christian pagans. But new work adds to evidence that they are the work of early Christians.


Ogham stones in Cork.

Some 400 Ogham stones have been found, ranging in size from meter-long ones to 2-ton stones up to 5 meters long. Damien McManus, a professor of Irish studies at Trinity College Dublin, has been scrutinizing a large collection of 28 newly cleaned and restored stones held at University College Cork. Like other Ogham stones, these have Celtic crosses etched onto them, and "the words have Latin endings," says McManus, who says the later stones have Latin as well as Ogham script. A reference to a priest on another stone suggests that all were etched by the earliest Irish Christians between the 5th and the 7th centuries, he says. Scholars believed that the crosses had been added later by Christians attempting to erase all pagan signs from their culture. McManus, who completed his analysis last month, believes the stones were carved not for rituals but to mark territory and burial sites.

Eamonn Kelly, keeper of antiquities at the National Museum of Ireland, says the new readings by McManus should convince most people that "the stones are certainly associated with the origins of Christianity in Ireland."

Hooke Home to Stay

"This is great news for science and great news for Britain."

—Martin Rees, president of London's Royal Society, which succeeded in purchasing—for $1.75 million—a recently discovered 17th century manuscript, containing Royal Society minutes recorded by famed physicist Robert Hooke, just before it was due to be auctioned off on 28 March.

HOPPERS AND CREEPERS TREED

A new amphibian family tree—probably the biggest phylogenetic tree ever completed for a class of vertebrates—was unveiled this month and has already attracted some carping from rival treemakers.

The project, detailed in 370 pages in the current issue of the *Bulletin of the American Museum of Natural History*, was instigated by Darrel Frost, an evolutionary biologist at the American Museum of Natural History in New York City. He and colleagues from more than a dozen institutions have spent the past 3 years comparing 1.8 million base pairs of DNA from 500 species in the three major amphibian groups: frogs, salamanders, and the earthwormlike caecilians. The analysis yielded 33 new groups and two new families of amphibians, says Frost. For example, a group of frogs ranging from South America to the American Southwest had to be dissolved, as some members proved to be most closely related to an Australian frog and others to American tree frogs.

"This study has just shed a floodlight on understanding how amphibians are related," says Claude Gascon of Conservation International in Washington, D.C. But others complain that the shufflings of amphibian relationships by Frost's team are based on subjective judgments that do not take enough tree-building strategies into consideration. "I think what they have done is irresponsible," says David Wake of the University of California, Berkeley. Such an effort "threatens to make taxonomy a laughingstock to other biologists." Wake is part of a National Science Foundation–funded amphibian tree project. Frost, whose effort was funded by NASA, agrees that there are debatable technical issues but suggests that "some people just don't like change."

AFRICAN SOIL EXHAUSTION>>

About 95 million hectares of arable land in Africa "have reached such a state of degradation that only huge investments could make them productive again," according to a new report from the International Center for Soil Fertility and Agricultural Development.

Agricultural productivity has declined in the past quarter-century in sub-Saharan Africa as its soils lose nutrients at the highest rate in the world. "I must tell you, the news is not good," said Amit Roy, CEO of the center, at a 30 March press conference in New York City. "Nutrient mining"—loss of soil nutrients through erosion, exhaustion by crops, and lack of fertilizer—is worst in East and Central Africa. Somalia is losing 88 kilograms of nutrients per hectare per year, says the report—compared with 9 kg in Egypt. African farmers desperate for fresh soil are clearing fragile forestlands and wildlife habitat.

Areas in red are where current population exceeds potential agricultural capacity.

Roy added that only 4% of arable land is irrigated, so water-supply problems also need to be addressed. The report will be presented at an Africa Fertilizer Summit to be held in Abuja, Nigeria, in June, where remedies such as cheap fertilizer, technical aid to farmers, and improved markets will be discussed.

CREDITS (TOP TO BOTTOM): TARAN GRANT/AMNH; PHOTO: WILDACRE/UNIVERSITY COLLEGE CORK; RONALD A. NUSSBAUM/UNIVERSITY OF MICHIGAN

Hitting a wall
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SOUTH KOREA

Premier Science University Ousts Unpopular President

SEOUL—An ambitious experiment to have an outsider reform South Korea's top science education institution has come to an ignominious end. Last week, trustees of the Korea Advanced Institute of Science and Technology (KAIST) in Daejeon voted not to renew the contract of its first non-Korean president, Robert B. Laughlin.

Two years ago, South Korea's science ministry recruited Laughlin, a Nobel laureate physicist from Stanford University, to transform the government-supported institution into one of the top-ranked universities in the world. Laughlin resolved that KAIST, focused largely on graduate training, should make radical changes. Among other recommendations, he proposed adding premedicine and prelaw departments and privatizing the university so it could charge tuition. Many faculty members opposed the measures, arguing that Laughlin's mandate was not to overhaul KAIST, but to raise funds and raise its profile. "Laughlin failed to carry out three terms in his contract: developing a vision for the school, making it a globally competitive institute, and attracting funding to invite finer students and professors," says physicist Choon-sup Yoon, head of KAIST's faculty association.

Parting shots. As the mystique of Stockholm faded, Nobelist Robert Laughlin (*left*) lost KAIST's support; he says he took the rap for systemic problems in Korean science.

Faculty members stepped up their criticisms earlier this year after Laughlin vowed to press ahead with unpopular reforms (*Science*, 20 January, p. 321). At a press conference organized by the faculty association on 23 March, several professors questioned Laughlin's integrity and leadership qualities, accusing him of being "dogmatic" and "impulsive." The association released a laundry list of derogatory statements about KAIST that it claims Laughlin made in lectures abroad and in meetings with foreign university heads. In mid-March, three of the university's four deans submitted letters of resignation, followed by the resignation of all 20 department chairs.

Presented with such overwhelming antipathy toward Laughlin, the 13 trustees present at the board meeting last week voted unanimously against a contract extension.

Laughlin says he is a victim of political circumstances, citing public distrust of scientists in South Korea in the wake of the scandal over disgraced cloning scientist Woo Suk Hwang. He dismisses the faculty reaction as "emotional" and characterizes ▶

U.S. SCIENCE POLICY

Revised NASA Media Rules Promise Greater Openness

The NASA scientist who said this winter that the agency had muzzled his views on climate change now says that he's "reasonably happy" with a draft media policy unveiled last week. But he and others worry about the rules' impact on whistleblowers.

Speak up. Griffin clarifies NASA policy.

On 29 January, *The New York Times* described complaints of harassment by James Hansen, director of NASA's Goddard Institute for Space Studies at Columbia University, including how a 24-year-old administrator with no scientific expertise had repeatedly blocked his efforts to share climate data and analysis with reporters. At the time, House Science Committee Chair Sherwood Boehlert (R–NY) assailed NASA's behavior as "wrong."

A few days later, the official was fired after it became clear that he had embellished his résumé. NASA Administrator Michael Griffin also ordered up an internal review of the agency's policies. On 30 March, Griffin released an eight-page document that says scientists "may speak to the media and the public about their work" as long as they give officials advance notice "whenever possible." Previously, scientists say, the unwritten policy was that employees needed to check first with public affairs officials.

Boehlert proclaimed himself "very pleased" with the revised policy and said he plans to monitor its implementation. Thomas Devine of the Washington, D.C.–based Government Accountability Project says that the rules fail to follow federal law on describing protection for whistleblowers, however, and that a vague description of what constitutes "sensitive but unclassified" data could give officials carte blanche in stifling dissent. Hansen says he's "a bit disappointed" by that ambiguity but that the new rules nevertheless represent "a huge change" for the better.

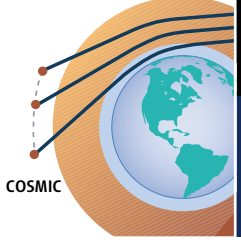
"The new policy does not establish any new restrictions," says a NASA spokesperson. The agency plans to finalize the policy in the near future.

–ELI KINTISCH

CREDITS (TOP TO BOTTOM): JONAS EKSTROMER/EPA/CORBIS; RENEE BOUCHARD/NASA

After the scandal 38

Education City, Qatar 46



A Cosmic turn 48

himself as a fall guy for shortcomings of the South Korean science system. "They can't hit government, so they hit me," he says. "My strategy for making KAIST better was to find things that were not good and expose them. Talking about the real problem is healthy. This unhappiness is the first step."

Some observers say that Laughlin's tenure was doomed from the outset. "The board made a mistake when they hired him. A lot of people knew it was a mistake, but Korea has a 'Nobel disease,' so a lot of people thought that someone with a Nobel Prize could fix anything," says Chung Wook Kim, a Korean-American physicist at Johns Hopkins University in Baltimore, Maryland. Although Chung calls Laughlin "a good physicist and a friend," he says Laughlin was "not qualified" to lead KAIST and that his "definition of reform was vague." Yoon emphasizes that the faculty is not allergic to reform: "Korean or foreign, if someone with statesmanship and management skills is appointed as president, we will support him or her."

Laughlin counters that his mandate was vague. "Everyone has different expectations, and … expectations got blurred," he says. The science ministry, meanwhile, has washed its hands of the affair. "What happens at KAIST is an internal issue," says a ministry official.

Laughlin plans to return to Stanford after his term ends on 14 July. His stature in South Korea may be diminished, but his bank account is not: He told *Science* that he accepted the KAIST presidency in the first place because the salary, approximately $500,000, was "too high to refuse." KAIST's future is less assured. If the trustees want reform, says Chung, they should appoint someone who is familiar with South Korean culture and politics.

–D. YVETTE WOHN

D. Yvette Wohn is a reporter in Seoul.

PALEONTOLOGY

Fossil Shows an Early Fish (Almost) out of Water

A fossil discovered in the upper reaches of Canada has revealed a creature that had the jaws of a fish, a head like a crocodile, fins better engineered for standing than swimming, and a future on land. This 375-million-year-old find, reported in the 6 April issue of *Nature*, is the latest—and most telling—fossil showing how aquatic vertebrates turned into four-legged landlubbers called tetrapods.

"It's the most significant discovery in years," says John Maisey, a fish paleontologist at the American Museum of Natural History in New York City. "It's got features that other fish don't have and features that tetrapods don't have."

Until now, the few fossils representing missing links between aquatic and terrestrial vertebrates have tended to be mostly fishlike or tetrapodlike instead of true intermediates. In 1999, paleontologists Neil Shubin of the University of Chicago and Ted Daeschler of the Academy of Natural Sciences of Philadelphia, Pennsylvania, happened upon a description in an undergraduate geology text of a large, isolated rock formation that was the right age to contain better representatives of this key transition. Canadian geological survey data indicated that these rocks were formed in a delta that emptied into an inland sea—a good environment for land-bound piscines.

For 4 years, the pair, along with Harvard zoologist Farish Jenkins Jr. and various students, traipsed north through the Nunavut Territory to field sites hundreds of kilometers from the nearest village or an airport. But they had little to show for their efforts until the first 3 days of their last season. Then, Shubin recalls, "we hit the mother lode." All told, the group has excavated 10 jawbones, three skulls, and two specimens in which the head and part of the trunk are in one piece—all of them belonging to a species the group calls *Tiktaalik roseae*, Inuit for "big freshwater fish."

Getting these treasures back to the labs intact was challenging, Shubin notes, and part of the biggest specimen had to be left behind. Daily sleet, snow, and cold weather also slowed the drying of the plaster casts needed to transport the rock. Colleagues are ecstatic that the group made such heroic efforts. "It's remarkably complete material," says Maisey. "We can see quite a bit of the anatomy."

Tiktaalik is not quite midway on the path to life on land, but close. Key tetrapodlike features have all but replaced fish traits, the researchers report. For example, until *Tiktaalik* came along, the earliest transitional fish still had gill cover plates, which pumped water over the gills, and its shoulder girdle was fused to the skull. *Tiktaalik* has no gill cover plates and just a few, reduced bones to tie the shoulder girdle to the skull. (Land vertebrates don't have any of these bones.) This fish therefore had a neck, concludes Daeschler. Also, he notes, it may have favored breathing air over underwater respiration.

Tiktaalik's head is flat with the eyes on top and the back of the skull squared off, much like the head of the earliest tetrapods. The fish does have the typical scales, but underneath them are overlapping ribs, which Jenkins had suggested 35 years ago would be needed to help support a body outside water.

The pectoral fins are lobelike, as expected, but their bones are like those in the arm and forearm, with hints of a wrist and hand at the very tip, says Jenkins. Novel joints observed in the fossil suggest that *Tiktaalik* could swing its fins out and forward, enabling it to push up and eventually lumber onto shore, says Shubin.

Landward bound. This ancient fish is a missing link between fish and land vertebrates.

Why head for the shore? These misfit fish, says Maisey, "could barely crawl on land, and they could barely swim." Still, the ability to flop out of the water likely helped them escape aquatic predators, and lifting their heads to look for land predators paved the way for descendants that abandoned their watery existence, he suggests.

The new fossil, says paleontologist Per Ahlberg of Uppsala University in Sweden, "goes a long way in filling one of the big gaps in the origin of tetrapods."

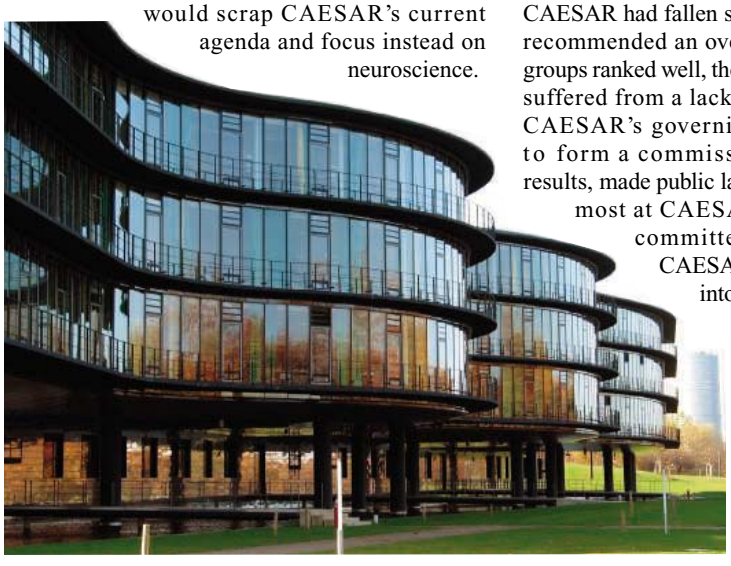
–ELIZABETH PENNISI

CREDIT: TED DAESCHLER/ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA

GERMAN SCIENCE

With Friends Like CAESAR's, Who Needs Brutus?

BERLIN—The perceived threat of a hostile takeover has researchers at the Center of Advanced European Studies and Research (CAESAR) worried for their jobs. The 7-year-old institute, which has 140 researchers in nanotechnology, bioelectronics, and medical imaging, received disappointing grades from a national evaluation team in 2004. Now Germany's Max Planck Society (MPG) has offered to take the institute under its wing. But the plan includes a bitter pill: It would scrap CAESAR's current agenda and focus instead on neuroscience.



New mission. The Max Planck Society has said the Center of Advanced European Studies and Research in Bonn should abandon nanotechnology and imaging research and focus instead on neuroscience.

CAESAR started as a consolation prize. When the reunified German government voted in 1994 to move its capital back to Berlin, the city of Bonn was awarded €1.4 billion ($1.7 billion) to turn itself into a "science city" (*Science*, 8 June 2001, p. 1827). CAESAR received a quarter of that sum as an endowment, with an ambitious mission to produce high-tech patents and spin off companies for the region. But in 2004, a harsh evaluation from the German Science Council (*Wissenschaftsrat*) said CAESAR had fallen short of expectations and recommended an overhaul. Although some groups ranked well, the report said, the institute suffered from a lack of focus. In response, CAESAR's governing board asked MPG to form a commission to advise it. The results, made public last month, were not what most at CAESAR had expected. The committee recommended that CAESAR be partially integrated into MPG as an institute for neuroscience, funded by the existing endowment. Current researchers on 5-year contracts would presumably not be rehired.

CAESAR scientists say that goes too far. "We are ready to sharpen our focus, but [the MPG plan] would mean simply giving up on everything we've done so far," says CAESAR spokesperson Francis Hugenroth. The institute has issued a formal reply, arguing that Germany already has a half-dozen top neuroscience institutes and that more than €15 million invested in materials science equipment would simply be written off. CAESAR also charges that patents and spinoff companies would be few and far between under the Max Planck plan.

Max Planck spokesperson Christina Beck says that far from a takeover, the MPG panel offered constructive suggestions for how to improve CAESAR's performance. "We have well-established, successful strategies" that govern many top institutes, she says. "That's why they asked us for advice." The panel decided that a focus on neuroscience would build on strengths at the University of Bonn and its research clinic, she says.

CAESAR researchers have at least one more chance to plead their case. On 12 April, the German Science Council's evaluation committee will hear presentations from CAESAR and from MPG. The overall council will then offer its public advice on 19 May. It would be hard to dismiss the Max Planck's plan completely, predicts Friedrich Tegelbekkers, head of the Science Council's evaluation committee, but "several details need to be discussed," including the prospects for industry spinoffs for the region. A decision from CAESAR's governing board is expected in June.

–GRETCHEN VOGEL

U.S. OCEAN POLICY

Major Fisheries Bill Introduced in House

Call it a net gain. Conservationists accuse the chair of the House Resources Committee of trying to water down environmental rules governing the management of U.S. fisheries. But the proposal by Representative Richard Pombo (R–CA) to reauthorize the 30-year-old Magnuson-Stevens Act would also elevate the role of science in fisheries management. And many environmentalists and scientists hope that legislators will tap elements of a second bill introduced last week to produce something more to their liking.

"I think we're moving in the right direction, but there are some things that make me nervous," says Andrew Rosenberg of the University of New Hampshire, Durham. Rosenberg and others say Pombo's bill (H.R. 5018) is less draconian than they had feared; they also like some of the features in a proposed bill (H.R. 5051) from Representative Wayne Gilchrest (R–MD), chair of the fisheries subcommittee, such as strict deadlines for ending overfishing.

The Magnuson-Stevens Act was last updated in 1996. Last fall, the Senate took up a measure (S. 2012) introduced by one of its namesakes, Senator Ted Stevens (R–AK), that garnered mixed reviews (*Science*, 25 November 2005, p. 1261), in part because fisheries managers would only need to consider scientific advice when setting limits on annual catches. Pombo's bill would require managers to follow the advice and, among other things, develop a procedure for peer-review.

But two other changes are setting off alarm bells. Pombo wants the secretary of commerce to have more discretion to extend a 10-year deadline for recovering overfished stocks. The secretary would also be able to exempt management plans from comprehensive analyses required under the National Environmental Policy Act (NEPA). "These are large loopholes," says Lee Crockett of the Marine Fish Conservation Network in Washington, D.C.

Conservationists prefer the bill by Gilchrest, which doesn't fiddle with NEPA or change the rebuilding timeline. Moreover, it also provides a mechanism to enforce annual catch limits, as does Stevens's bill. In addition, Gilchrest calls for a new 1-year deadline to cease overfishing of any stock identified as depleted. Scientists also like a provision that would require the National Oceanic and Atmospheric Administration to develop guidelines for ecosystem-based management of fisheries.

A staffer for Gilchrest concedes that Pombo's leadership post allows him to call the shots on the reauthorization. But Gilchrest hopes that some of his provisions will be folded into whatever bill moves forward. No House hearings have been scheduled; Stevens's bill is awaiting floor action.

–ERIK STOKSTAD

CREDIT: CAESAR

CHEMISTRY

New Polymer May Rev Up the Output Of Fuel Cells Used to Power Cars

ATLANTA, GEORGIA—Most technologies must keep constantly improving to stay on top. But in the world of low-temperature fuel cells—the sort used to power cars—a polymer membrane made by DuPont, called Nafion, has been the gold standard for decades. Last week, however, at a meeting here of the American Chemical Society, researchers from North Carolina unveiled an upstart that might finally dethrone Nafion and markedly improve the performance of automotive fuel cells.

Fuel cells work by converting chemical fuel directly into electricity without burning it. The standard approach requires reacting hydrogen and oxygen at two different electrodes separated by a thin plastic membrane. At one electrode, hydrogen molecules are stripped of their electrons, which are then sent through an external circuit to do work. The leftover protons are channeled through the polymer membrane to another electrode, where they meet up with oxygen and the circulating electrons to produce water.

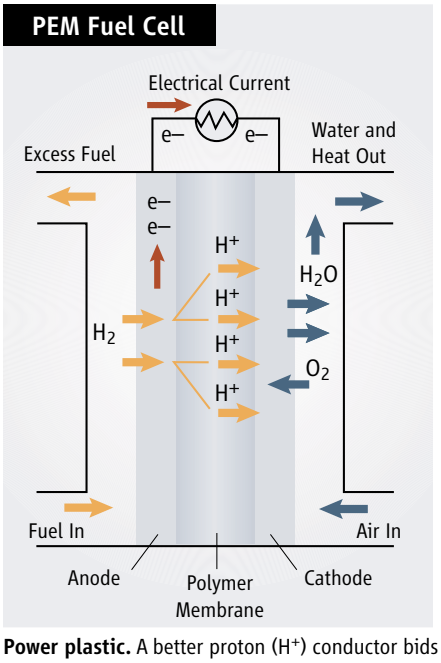
But making good proton conductors from polymers isn't easy. Nafion's strategy is to link acid groups to the end of fluoropolymer chains. Because acids hold on to their protons only loosely, they are good proton conductors. But acids also readily dissolve in water, which is needed for standard proton exchange membrane (PEM) fuel cells to operate. So if you put too many acids on your polymer, it falls apart when you run your fuel cell.

In hopes of boosting the acid content of their polymers, Joseph DeSimone, a chemist at the University of North Carolina, Chapel Hill, and his graduate student Zhilian Zhou decided to create a polymer with extra links between the chains so that it wouldn't dissolve in water. They started with a heavily fluorinated polymer called perfluoropolyether, which they copolymerized with a derivative of an acid-rich compound called styrene sulfonic acid. The researchers mixed the compounds as liquids and then polymerized them using ultraviolet (UV) light. The UV light knitted the two compounds together into chains and forged links between the chains, creating an extended network of polymers that doesn't dissolve when the water content climbs.

Because the polymer contained more acid groups, it conducted protons nearly three times as well as Nafion. "This sounds very nice and could set a new gold standard," says Robert Hockaday, a fuel cell expert who runs Energy Related Devices Inc., a fuel cell company in Los Alamos, New Mexico.

Unlike Nafion, which comes only in thin sheets, the new polymer also can be cured from its liquid precursors in essentially any shape. Zhou and DeSimone, for example, patterned their polymer using a standard stamping technique into a form with a much higher surface area than a flat film. That surface area is key to fuel cells because engineers pattern the two sides of their films with the metals, such as platinum, that make up the electrodes that carry out the needed chemical reactions. An increased surface area allows for a more widespread platinum coating, leading to increased power. In this case, the patterning doubled the power output of their fuel cells.

The new polymers could bring another advantage as well. Because the crosslinked polymers are likely to be far more robust than Nafion, they should withstand higher operating temperatures. That's key, because raising a fuel cell's operating temperature from the standard 80ºC to about 120ºC ought to prevent contaminants such as carbon monoxide from glomming onto the platinum catalyst and sapping the cell's performance. DeSimone says he expects that their new crosslinked polymer membrane will hold up far better than Nafion at higher operating temperatures, but he and Zhou haven't had a chance to run the experiment. Their continued success could herald a new king of the hill among polymer fuel cells. **–ROBERT F. SERVICE**



Power plastic. A better proton (H^+) conductor bids to unseat the longtime champion at the heart of large fuel cells.

CREDIT: ADAPTED FROM U.S. DEPARTMENT OF ENERGY

Maryland Goes for Stem Cells

Maryland is about to become the fourth state—after New Jersey, California, and Connecticut—to create its own human embryonic stem cell research program after Republican Governor Robert Ehrlich pledged to sign a bill passed last week by state lawmakers.

The bill, a 5-year authorization that sets up a commission to oversee the work, is the result of some fancy footwork by the Democrat-controlled legislature that avoids any mention of "human embryos" and substitutes the term "material." But it defines stem cells as cells that "divide indefinitely" and give rise to "many" cell types, thus excluding most adult stem cells. The bill also outlaws reproductive cloning. Although it doesn't forbid research cloning, it authorizes funds only for research on embryos that would otherwise be discarded by fertility clinics. A separate spending bill included $15 million for the work next year.

Curt Civin, a stem cell researcher at Johns Hopkins University in Baltimore, welcomes the law, saying Maryland's investment might eventually be "comparable" to the $300-million-a-year California program on a per capita basis. Supporters say they expect additional state funding if Ehrlich loses his reelection bid in November, as Republican lawmakers whittled down an initial plan to spend $25 million. **–CONSTANCE HOLDEN**

Collateral Damage

TOKYO—A genetics center is the latest victim of a scandal roiling the Japanese scientific community. Last week, the National Institute of Advanced Industrial Science and Technology in Tsukuba announced it will close a 3-year-old gene function research center led by Kazunari Taira, a University of Tokyo chemist under fire over his failure to substantiate findings in a series of papers published in prominent journals (*Science*, 3 February, p. 595).

In an action that an institute official says is not a disciplinary measure, the institute has opted not to extend the contracts of Taira and a key associate, transferring the 51 other center staff members to other labs. "The misconduct problem has made it very difficult to administer the research center," says institute director Masanori Yoshikai. Last week, a University of Tokyo investigative committee said it found no solid evidence of deliberate fraud in the original work but that Hiroaki Kawasaki, a research associate in Taira's lab, fabricated data during attempts to reproduce the experiments. Taira intends to retract four papers; a disciplinary panel will weigh in next week.

–HIROMI YOKOYAMA

DEVELOPMENT AND ECOLOGY

Villagers Drafted Into China's Model of 'Sustainability'

HUANGBAIYU, CHINA—Nestled in jagged, snow-clad hills near the North Korean border, the 42 unfinished gray houses laid out in neat rows have all the glamour of a trailer park. But the cookie-cutter single-story homes of Huangbaiyu, or "Yellow Cyprus Valley," are the nucleus of China's first "sustainable village."

Socialist paradise? Huangbaiyu should free land for sowing crops and reforestation—if Dai Xiaolong can persuade villagers to move in.

Proponents say that the new Huangbaiyu, set to open this summer, marks the start of a revolution: a path for China to slash its voracious consumption of natural resources and convert wasted rural space into croplands or forests. Huangbaiyu's houses are made largely from renewable materials, and residents will cook with gas distilled from agricultural waste. "It's a good concept," says Guofan Shao, a forest ecologist at Purdue University in West Lafayette, Indiana, who has conducted research near Huangbaiyu but is not affiliated with the project.

The experiment's timing could not be more propitious. Alarmed by a growing divide between rich cities and impoverished villages, Chinese leaders at the annual meeting of the National Peoples' Congress last month gave marching orders to improve rural conditions. Huangbaiyu's village committee director, an entrepreneur named Dai Xiaolong, wants to spread the "brand" to every corner of China. That ambition unsettles some observers, who fear that indiscriminate adoption of the model could line the pockets of a handful of developers while blighting the landscape. It's also not clear whether villagers will voluntarily move from dwellings scattered in a several-kilometer radius into their new digs.

The author of the village concept is Deng Nan, a daughter of former premier Deng Xiaoping. Responding to Deng's overtures, former U.S. President Bill Clinton in 1999 tapped architect William McDonough to be U.S. co-chair of a newly created China-U.S. Center for Sustainable Development, a nonprofit organization in Portland, Oregon. (Deng, a former vice-minister for science, is China's co-chair.) The winning proposal was submitted by Huangbaiyu, southeast of Benxi, a polluted industrial city.

The Appalachia-like landscape, pocked with open-pit mines and marred by denuded hills, is ripe for revitalization. Life is hard for the valley's 1400 denizens, most of whom subsist on growing corn, raising Cashmere goats, and farming rainbow trout; they earn on average $440 per year.

At the demonstration village site, the smell of burning coal fills the air. The project is designed to make life more comfortable as well as improve energy efficiency. The new homes are made from compressed earth blocks using technology from Vermeer Manufacturing; BASF has donated roof insulation; and BP solar panels are being tested on two homes. "We will raise the living standard of the peasants," Dai says. As a measure of optimism, each house has a one-car garage; few residents currently own cars.

Around 400 houses are planned, enough to accommodate virtually the entire present population. As families move to new homes, their scattered former dwellings will be demolished. "We have very limited land resources, so reuse is very important," says Benxi mayor assistant Dai Limin. Dai is a senior researcher with the Institute of Applied Ecology of the Chinese Academy of Sciences in Shenyang, which will monitor the land reclamation. Concentrating villagers in a single residential area, says Shao, should allow managers to improve the health of the mixed pine and broadleaf forest.

The benefits are less obvious to Huangbaiyu residents. Shannon May, a graduate student in anthropology at the University of California, Berkeley, who has been living here for the past year, says many residents worry that they may have to forfeit access to land near their current homes where they rear trout or graze goats. Local officials may feel compelled to resort to "top-down coercion to make people move" before the China-U.S. center's board visits Huangbaiyu in June, May says: "The next few months will be a pretty anxious time for everybody involved."

In the meantime, other regions, including Beijing and Shanxi, are contemplating similar projects. For China's 900,000 villages, Huangbaiyu's experience may be the first step toward a distant dream of sustainable development—or a dystopian nightmare.

–RICHARD STONE

MEDICINE

Old Drug, New Hope for Marfan Syndrome

People with Marfan syndrome live with a ticking bomb. Their aortas, unless surgically replaced, gradually enlarge and weaken until they fatally rupture. But prompted by a new explanation of what causes Marfan syndrome, pediatric cardiologist Harry Dietz of Johns Hopkins University in Baltimore, Maryland, and his colleagues may have come up with a surprising tool to defuse this lethal situation: losartan, a drug already approved in the United States for use against high blood pressure. On page 117, they report that in a mouse model of Marfan syndrome, the drug prevents aortic aneurysms as well as lung problems sometimes seen in the condition.

"It's a beautiful story. It's one of the most classic examples of translational science I've seen in the cardiovascular arena," says Kenneth Chien, director of the Massachusetts General Hospital cardiovascular research center in Boston. The study, he adds, "makes a very compelling case" that losartan should be tested immediately in people. In fact, Dietz's team has begun administering the drug to a few children with a severe form of Marfan syndrome who have rapidly deteriorating aortas. The National Institutes of Health (NIH) plans to start a large trial of the drug as soon as this fall.

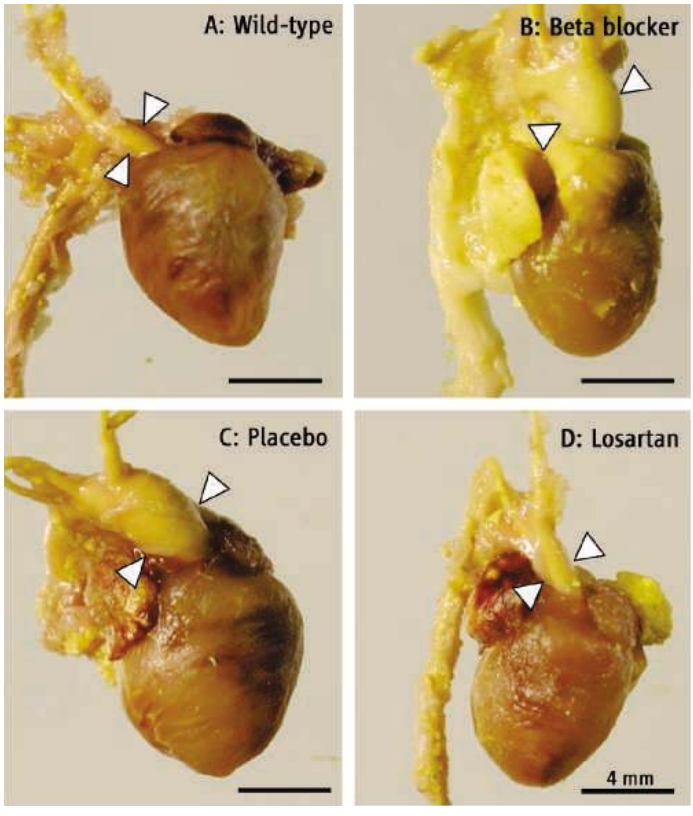
This enthusiasm is a far cry from the pessimism that has plagued the Marfan field. Experts once thought that a structural defect in connective tissue led to the aortic aneurysms, lung problems, and other features of Marfan syndrome. In 1991, Dietz and other researchers had reported that mutations in the gene encoding fibrillin-1 are responsible for the syndrome. This protein forms fibrils in the matrix outside cells, so the mutations were thought to rob elastic tissue of a key building material. The only apparent way to treat ▶

CREDIT: R. STONE

such a defect was to restore the missing fibrillin-1, yet gene therapy and other potential replacement solutions seemed like distant prospects.

Almost from the outset, Dietz had doubts about this explanation for Marfan syndrome. "We began to recognize that selective features couldn't be explained by disease models that relied on weakness of tissues," the Howard Hughes Medical Institute investigator says—bone overgrowth, for example, was difficult to reconcile.

In 2001, Dietz's team reported creating mice that make low amounts of fibrillin-1 and develop emphysemalike lung problems similar to those seen in about 10% of Marfan patients. But a close look at the mouse lungs showed that the defect began early in development, challenging the notion that the emphysema was due to the deterioration of lung tissue over time. The scientists instead fingered overactivity of a protein called transforming growth factor–β (TGF-β); they also found that administering antibodies to it prevented the lung damage in the mice.



Heart of the matter. The aorta (arrows) of a normal mouse (A) and a losartan-treated mouse with a fibrillin-1 mutation (D) are indistinguishable, but those of mutant mice treated with a beta blocker (B) or placebo (C) have aneurysms.

Dietz now believes that fibrillin-1 binds TGF-β in the extracellular matrix, keeping it inactive. In people with Marfan, the growth factor is unleashed, he hypothesizes. Indeed, Dietz and his group have also shown that antibodies to TGF-β can prevent heart valve problems and aortic aneurysms in their Marfan-like mice.

Seeking a more practical way than antibodies to block TGF-β signaling, the researchers hit upon losartan, which was known to somehow thwart the growth factor. It seemed a natural drug to explore because many physicians treating Marfan syndrome already prescribe blood pressure–lowering drugs called beta blockers to ease stress on the aorta and slow its growth.

For their losartan study, Dietz's group worked with mice engineered to have a fibrillin-1 mutation analogous to those seen in Marfan syndrome. They began administering the drug to 2-month-old mice, which already have obvious aortic changes. After 6 months, the aortic aneurysms had worsened in mice given either a placebo or a beta blocker, but normal mice and losartan-treated Marfan ones were indistinguishable, indicating that the drug had reversed the early aorta damage. "It was truly a jaw-dropping moment. It was beyond anything I could have anticipated or hoped," says Dietz.

Because the drug has a good safety record in people, Dietz and his colleagues are already giving losartan to a few children. But he cautions that there could be unanticipated side effects in those with Marfan syndrome. These initial tests on the children with rapidly progressing aneurysms are ethically defensible, says medical geneticist Peter Byers of the University in Washington, Seattle, because the children will otherwise need multiple, risky surgeries as they outgrow initial aortic replacements.

Dietz says NIH is also finalizing plans to enroll 700 to 1000 people with Marfan syndrome, ranging from 6 months to 25 years old, in a trial coordinated by a multicenter group called the Pediatric Heart Network. Patients will receive either losartan or a beta blocker. Byers notes that the "striking" mouse results could make it difficult to recruit patients into a trial in which they may not receive losartan.

Meanwhile, Dietz and his colleagues are looking into whether losartan can treat additional conditions associated with aortic aneurysms. "I think you are going to see this [drug] extended across diseases that are TGF-β–related," predicts Chien. **–JOHN TRAVIS**

CREDITS (TOP TO BOTTOM): J. HABASHI *ET AL.*, *SCIENCE*; U.S. DOE

Italian Voters Ponder Science

A hot topic in Italy's election on 9 and 10 April is whether research has thrived under Silvio Berlusconi's conservative government. His research minister, Letizia Moratti, has installed a commission for evaluating research, centralized academic recruitment, and secured more private funding for national institutes, aligning them with national goals (*Science*, 1 April 2005, p. 35). Berlusconi insists the moves will raise competitiveness, but the left-wing opposition and many in the research community say the policies have weakened Italian science.

At a rally in Rome last week, the left-wing coalition led by Romano Prodi announced that, if elected, it would more than double Italy's rate of research spending from the current 1.1% to 3% of gross domestic product by 2010.

–SUSAN BIGGIN

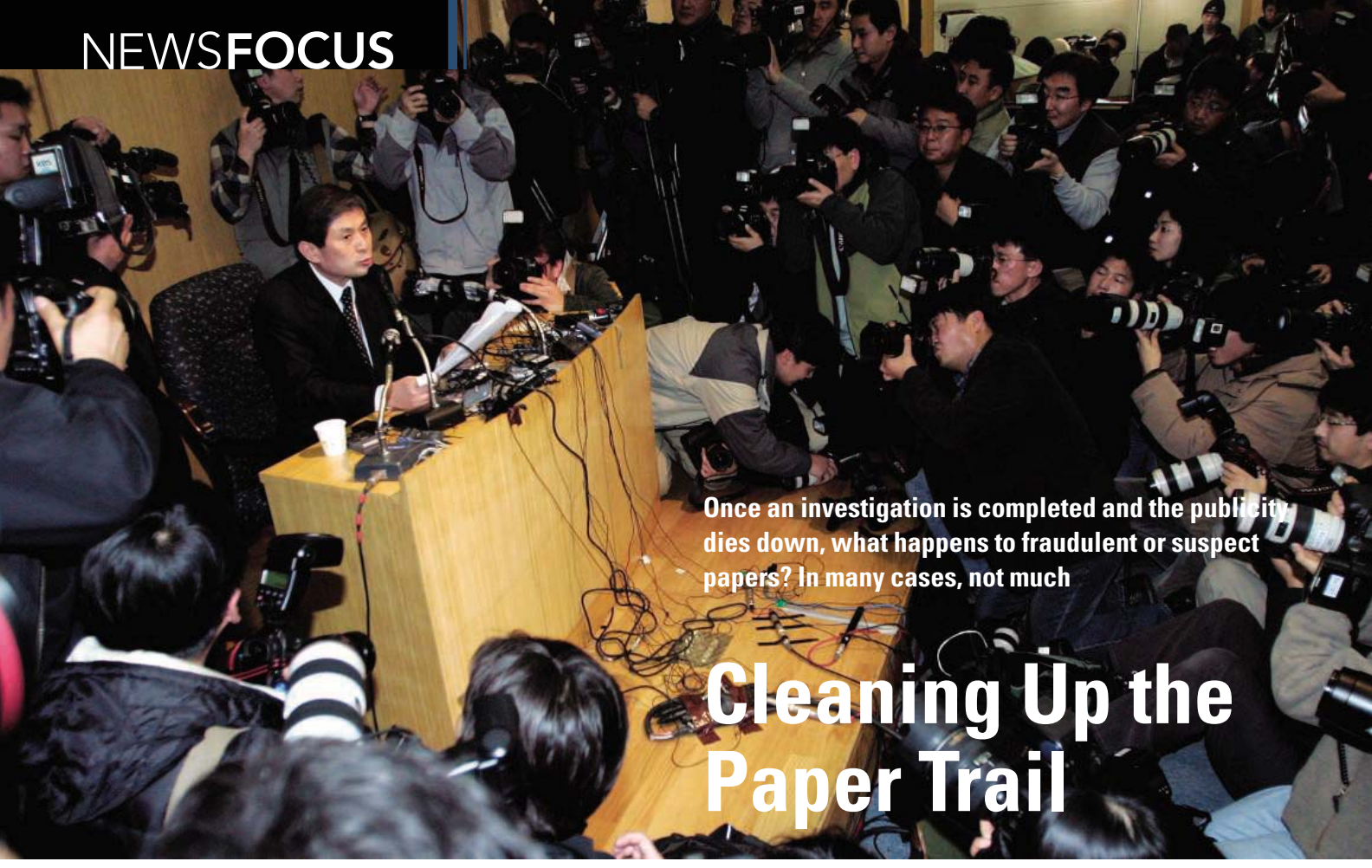
Updates

■ After a slow start, a U.S. advisory panel set up to prevent the results of federally funded biology research from being used by terrorists has put out two draft proposals. The National Science Advisory Board for Biosecurity has aired a report on so-called dual-use experiments and drafted guidelines to help journals screen papers.

■ India has joined the government steering committee of a U.S. project to build a $1 billion advanced coal plant that sequesters carbon dioxide and produces hydrogen (design below). Meanwhile, 22 sites in nine states have said they plan to compete for the plant, called FutureGen. Proposals are due 4 May.

■ Last week, the U.S. Supreme Court heard the second set of arguments in 2 weeks on patents. The cases involve patenting a scientific concept and the power of a patent to halt a competitor (*Science*, 17 February, p. 946). The biotech community is closely awaiting the decisions, expected by June.

■ Ending a 2-year battle, France's National Assembly this week gave final passage to a research reform bill. Researchers are disappointed that the bill offers no guarantees that science budgets will be indexed for inflation until 2010 (*Science*, 24 March, p. 1693).



Once an investigation is completed and the publicity dies down, what happens to fraudulent or suspect papers? In many cases, not much

Cleaning Up the Paper Trail

LAST OCTOBER, AFTER A 14-MONTH investigation, immunologist Luk van Parijs was fired from the Massachusetts Institute of Technology (MIT) in Cambridge. The school alleged that he had confessed to faking data in one published paper, several unpublished manuscripts, and grant applications.

Van Parijs's academic future may be shot to pieces. But his scientific past, so far, is intact: roughly 40 papers stretching back to 1994, many of them in the blossoming field of RNA interference. None has yet been publicly labeled fraudulent or retracted. MIT has not said which paper it found to be problematic. Other investigations are continuing.

"One of the biggest problems in these fraud things," says Kathleen Case, publisher at the American Association for Cancer Research (AACR) in Philadelphia, Pennsylvania, is that "the investigations get finished, the wrist-slapping [ensues]. And the last thing people think of is the journals." AACR publications ran three of van Parijs's papers.

An examination by *Science* of more than a dozen fraud or suspected fraud cases spanning 20 years reveals uneven and often chaotic efforts to correct the scientific literature. Every case has its own peculiarities. Whether wayward authors confess to fraud; whether investigations are launched at all, and if they are, whether their scope is broad or narrow; whether fraud findings are clearly communicated to journals—each of these helps determine how thorough a mop-up ensues.

Large-scale fraud cases are rare. But scientists whose work is challenged have often co-authored dozens or even hundreds of papers. How their legacy is handled may determine whether work by innocent co-authors, particularly young scientists, is wrongly tainted. But debates rage over how comprehensive fraud investigations need to be—whether, for example, they ought to examine a scientist's entire body of work regardless of expense.

"The responsibility is very much on the shoulders of those who know [of fraud] to correct the record as speedily as possible."

—Francis Collins
Director, National Human Genome Research Institute

And then there are the journals, keepers of the historical record. Journal editors often stress—and universities and funders agree—that publications are in no position to investigate fraud. The burden, they say, should be on institutions and funding agencies; they have the money and staff to convene sweeping inquiries and demand raw data. Traditionally, journals wait for the results of inquiries to steer decisions on problem papers. Some act only if a retraction has been requested by a paper's authors—preferably all of them. But authors accused or suspected of fraud often don't agree to a retraction. Editors must then make a potentially career-wrecking decision, with varying degrees of guidance.

Even papers that investigators have found fraudulent can linger in the scientific record for years. In one case, findings of a fraud investigation in Germany were not translated into English. In another, some journals declined to correct obesity papers that a U.S. agency's exhaustive inquiry had deemed partly fake.

Fear of being sued lies behind inaction in some cases, especially when there has been no clear-cut finding of fraud. Some journals, however, are becoming more assertive, contacting investigators and settling on their own middle ground in nebulous cases. "All the participants are making up the rules as we go along," says Barbara Cohen, executive editor of the *Public Library of Science* (*PLoS*) Publications.

Mopping up

A researcher is found guilty of fraud. A black mark is splashed across certain published papers, and it's recommended that they be withdrawn. What happens next?

CREDIT: KIM KYUNG-HOON/REUTERS

What now? Several papers by Korean cloner Woo Suk Hwang and his team have already been retracted, but who will validate or remove the rest?

"The responsibility is very much on the shoulders of those who know [of fraud] to correct the record as speedily as possible," says Francis Collins, director of the National Human Genome Research Institute in Bethesda, Maryland. In the mid-1990s, one of his graduate students, Amitav Hajra, confessed to faking data on leukemia projects. In that case, all the authors requested that three papers be retracted and two others corrected. The journals responded within months.

For the journals, a confession followed by author unanimity to pull a paper is a best-case scenario. "The official rule for journals is that the authors must do the retracting," says AACR's Case. A retraction on these terms sharply reduces the legal risk that journals will be accused of tainting a scientist's reputation by retracting a paper without his or her consent.

What to do when an alleged fraudster doesn't confess is fuzzier. "More and more, … the authors dig in their heels and try to salvage some of their reputation," says Case. When this happens, journals often rely on the findings of investigators.

And here, they often hesitate.

Last March, for example, the Office of Research Integrity (ORI), which was formed in 1989 to investigate misconduct cases involving funds from the National Institutes of Health (NIH) and certain other federal agencies, broke bad news to 10 publications: a paper they had published was fraudulent.

The news was not wholly unexpected. Eric Poehlman, an obesity and aging researcher at the University of Vermont in Burlington, had left the school after a whistleblower brought concerns of research inconsistencies to university officials. ORI oversaw its biggest inquiry ever, covering 10 papers co-authored by Poehlman and 15 of his NIH grant applications. All 10 papers, they determined, contained fabricated data and ought to be retracted or corrected.

An ORI finding, many journal editors say, gives publications ironclad backing to withdraw a paper even if an author doesn't cooperate. But ORI officials weren't happy with the journals' response in the Poehlman case. By last September, 6 months after the office issued its report, six of 10 journals had published retractions or corrections, supplied by Poehlman as part of his agreement with government officials. Two more followed. But two journals have not acted at all, according to ORI officials and journal records. (Poehlman has pleaded guilty to making false statements on a federal grant application and is awaiting sentencing.)

The spotty response in the Poehlman case echoes another from 2 decades ago. In the mid-1980s, Paul Friedman, a radiologist and then–associate dean of the University of California, San Diego, spent 15 months overseeing an investigation of 135 publications by a colleague, Robert Slutsky, who was accused of widespread fraud. Of the 60 publications judged fraudulent or questionable, *Science* found retractions for 18. "The journals responded very variably," says Friedman.

Journal editors, however, say the situation is rarely black and white. In the Poehlman case, the two journals that haven't carried out ORI's recommendations are outside the United States. Their editors may be less familiar with ORI, although ORI officials have no evidence that this explains their inaction.

Lengthy inquiries and garbled communication can also complicate removing tainted papers. Both were on vivid display in the case of cancer specialist Friedhelm Herrmann, who worked in Berlin, Freiburg, and Ulm, Germany.

> Investigating 100 papers **"is a horrendous investment."**
> —Alan Price, Office of Research Integrity

CASE FILE

Name: Eric Poehlman
Age: About 50
Institution: University of Vermont College of Medicine
Discipline: Menopause, aging, and obesity
Paper Trail:
Author or co-author of 204 papers
10 papers found with falsified data, to date
8 papers retracted or corrected
Current status: Pleaded guilty to making false statements on a federal grant application. Awaiting sentencing.

FRAUD INVESTIGATION
Began: 2000
Status: Still ongoing
Run by: U.S. Office of Research Integrity and the University of Vermont

Russell Tracy, Leader of university investigation

In 1998, Ulf Rapp, a cancer researcher at the University of Würzburg in Germany agreed, with some trepidation, to lead an inquiry into Herrmann's work set up by the DFG, Germany's main science funding agency. Rapp and his helpers painstakingly examined hundreds of autoradiograms, images that reflect RNA and protein production. In paper after paper, they uncovered autoradiograms that had been manipulated, flipped upside down, or recycled from earlier experiments. The investigators identified 29 "falsification-beset" publications; 28, they concluded, "should be withdrawn," and in one case, a correction "would be sufficient." Another 65, nine of them book chapters, were deemed "strongly suspicious."

The investigation took more than 2 years, during which editors came and went at many journals. "What may have been on one person's radar isn't going to show up on another person's," says John Hawley, executive director of the American Society for Clinical Investigation, which publishes the *Journal of Clinical Investigation*. *JCI* issued four retractions of articles co-authored by Herrmann, but three appeared in 2003, years after retractions elsewhere. (The fourth was retracted in 1998 at the behest of two authors.) *JCI*, says Hawley, was unaware that some other journals were retracting Herrmann's work. "But of course," he says, "we will become aware of something eventually."

In the Herrmann case, the DFG notified some journals directly, says DFG spokesperson Eva-Maria Streier, although none with whom *Science* spoke could recall having heard from the agency. The DFG "got cold feet," says Rapp, because Herrmann vigorously denied fraud and threatened to sue. The DFG posted Rapp's report

PHOTO CREDIT: RAJ CHAWLA/UNIVERSITY OF VERMONT MEDICAL PHOTOGRAPHY

on its Web site, but, Rapp recalls, it wasn't easy to find there.

Streier responds that the DFG distributed the report at a press conference and posted it online. The list of papers was in English, she says, although she acknowledges that blurbs summarizing the fraud status of those publications went untranslated.

At the journals, the result was chaos. AACR journals had published six papers co-authored by Herrmann that Rapp's inquiry deemed fraudulent or suspicious. Case, who never saw the DFG report, contacted Rapp. He recommended the papers be withdrawn. But "this is one guy saying you probably should," says Case. All six papers are still in the literature.

"I think we got a preliminary report … that was written in German" but couldn't glean a clear message from it, says Richard Dodenhoff, journals director of the American Society for Pharmacology and Experimental Therapeutics, which publishes *Molecular Pharmacology*. Like other editors, he says he traditionally waits for guidance from investigators. "Normally, we rely on some official body to tell us that there's something wrong," says Dodenhoff, adding that the journal was never informed that its papers should be pulled. The journal has three publications on the "falsification-beset" list and one on the "strongly suspicious" list. None was retracted.

Other journals reacted differently. *Blood*, a popular venue of Herrmann's, was well aware of the investigation early on. The editor at the time, Kenneth Kaushansky, a hematologist at the University of California, San Diego, retracted eight articles by Herrmann, all of those it knew to be problematic, over protests from the journal's attorney that the corresponding author—in several cases, Herrmann himself—had not given permission.

> "All the participants are **making up the rules as we go along.**"
>
> —Barbara Cohen, Executive Editor, *PLoS* Publications

Finally, fraud investigators propose another reason why journals sometimes might be reluctant to pull a paper: Retractions may rank low on the priority scale and can breed bad blood between the journal and researchers. They can also reflect poorly on a publication. "You don't want to make waves," says Friedman. The journals with which he corresponded in the Slutsky case ranged from pleasantly collegial to downright defensive.

Rapp's contact with the journals left a bitter taste in his mouth. Most ignored his notes and faxes, he says, or "wrote back very nasty letters." Several, he says, remained determined to get permission from every author on the paper. The reaction "did surprise me. … It seemed to me we were helping those guys. They had a rotten egg in their basket. We gave them a chance to clear it up."

Casting the net

If a critical question for journals is whether to retract papers, the typical bind for investigators is how wide a net to cast after a paper is alleged to be fraudulent. The spectrum of opinions here is vast. An investigation's breadth lays the groundwork for how much might be corrected, and how much collateral damage—papers by a suspect researcher left unexamined by investigators—will remain.

Some investigators are driven by sheer curiosity and a desire to get at the truth. Uncovering some fraud leaves the nagging question: "You wonder, is any of his previous work, and there was a lot of it, invalid?" says Friedman, explaining the investigation of Slutsky, which examined every one of the radiologist's papers.

But is it worth parsing a 10- or 20-year-old paper when this saps time and money from university faculty members, government officials, and journal editors? Some scientists and journals favor digging into every paper by a known or suspected fraudster, whereas others believe that narrower inquiries suffice. Often, there is no easy way to measure the value of catching fakery in the scientific literature.

Even Retracted Papers Endure

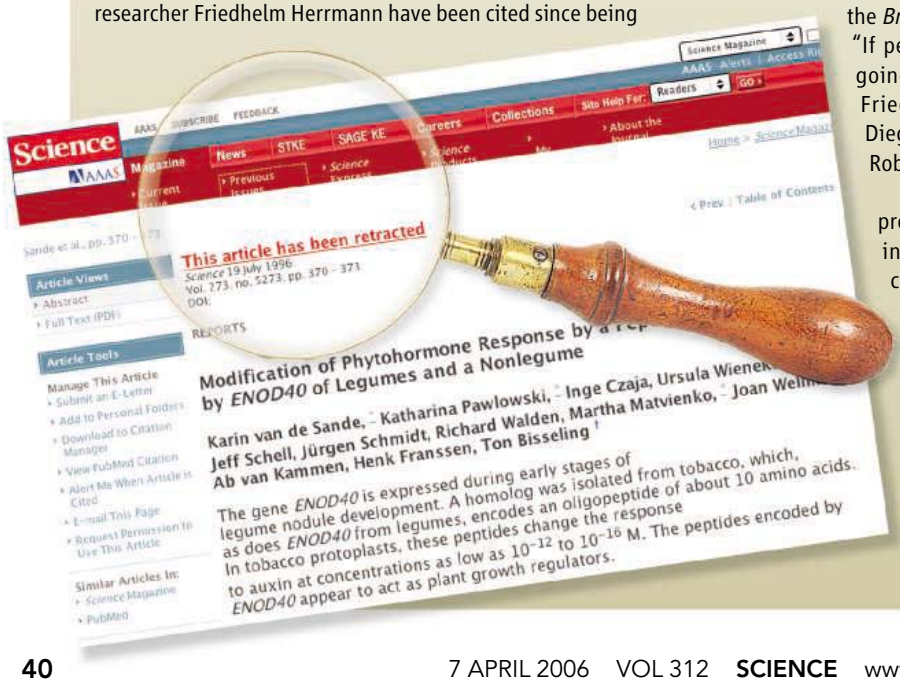
Like ghosts riffling the pages of journals, retracted papers live on. Using Thomson Scientific's ISI Web of Knowledge and Google Scholar, *Science* found dozens of citations of retracted papers in fields from physics to cancer research to plant biology.

Seventeen of 19 retracted papers co-authored by German cancer researcher Friedhelm Herrmann have been cited since being retracted, in some cases nearly a decade after they were pulled. Together, two of those papers were cited roughly 60 times. Examination of one *Nature* paper by former Bell Labs physicist Jan Hendrik Schön, published in 2000 and retracted in 2003, revealed that it's been noted in research papers 17 times since, although the drop-off after retraction was steep: Prior to being pulled, the paper was cited 153 times.

It's "quite embarrassing," says Richard Smith, former editor of the *British Medical Journal*, of references to retracted publications. "If people cite fraudulent articles, then either their research is going to be thrown off or something will be wasted," says Paul Friedman, a former dean at the University of California, San Diego, who oversaw an investigation into papers by radiologist Robert Slutsky in the mid-1980s.

In some cases, citations are "negative": The paper is cited precisely because it was retracted, and the retraction duly noted in the text. But those familiar with postretraction citation consider that rare. "It almost never happens," says Drummond Rennie, a deputy editor of the *Journal of the American Medical Association*. Spot checks of 10 papers that cite withdrawn publications found no negative citations.

Instead, scientists often don't know that the work they are citing has been retracted. Lon Kaufman, a cell biologist at the University of Illinois, Chicago, was surprised to learn from *Science* that his 1999 article in *The Plant Journal* cited



Afterlife. This *Science* paper was retracted nearly 7 years ago, but that hasn't stopped other researchers from citing it.

CREDIT: (OVERLAY) CLIPART.COM

For people like Steven Shirey, that value is incalculable. Last July, the Washington, D.C., geologist had a 2-cm by 2-cm square of skin excised from his tongue. The experience was traumatic enough, but Shirey felt he had no choice: Genetic testing had revealed that a lesion found there was aneuploid, meaning it had an abnormal number of chromosomes. A Norwegian oncologist, Jon Sudbø of the University of Oslo, had found that 84% of people with aneuploid lesions go on to develop a deadly form of oral cancer. The work appeared in 2001 and 2004 in *The New England Journal of Medicine* (*NEJM*).

"Based on Sudbø's article, I thought I had a death sentence," Shirey says, adding that he would have signed up for a preventive chemotherapy trial had one been available.

In January, after a whistleblower raised questions about data in a recently published *Lancet* paper, Sudbø admitted through his attorney to faking signature findings on aneuploid lesions in the 2004 *NEJM* paper. The 2001 paper is under suspicion after journal editors found that it contains a pair of duplicate images. Anders Ekbom, a surgeon at the Karolinska Institute in Stockholm, is leading an investigation into all of Sudbø's 38 research papers. He hopes to complete it later this spring.

Shirey knows now that one of the publications that guided him as a patient contained faked data (although its conclusions may turn out to be correct). But his story is a cautionary tale of the devastating impact fraudulent findings can have. Of all types of fraud, fakery in clinical research tends to engender the greatest sense of urgency among universities and investigators. In its breadth, the Sudbø inquiry mirrors unusually comprehensive inquiries in two other fraud cases, those of Herrmann and Poehlman. All encompass clinical research that can directly affect patients—and in some instances, already has.

> "Over some measure of time, **the community … has a way of self-correcting.**"
>
> —Samuel Kaplan
> University of Texas, Houston

Even in cases like these, though, there's disagreement over the hours, money, and sweat worth pouring into an investigation. ORI prefers extensive inquiries and frequently prods institutions to expand them, says Alan Price, ORI's associate director for investigative oversight. But there comes a point at which even ORI feels it has to stop. Investigating 100 papers "is a horrendous investment," says Price. The inquiry into 10 papers and 15 grant applications of Poehlman's took the better part of 2 years. Poehlman's name is on 204 papers in all. "At some point," says Price, "you have to say, 'Maybe this implies that his work cannot be trusted.' "

But Poehlman's employer, the University of Vermont College of Medicine, isn't satisfied. After ORI's findings on Poehlman were released a year ago, "we were left with nothing said about the vast majority of his 200-odd papers," says Russell Tracy, senior associate dean for research and academic affairs. The school, says Tracy, felt an obligation to those who participated in Poehlman's menopause studies, "and responsibility to the people of the country whose clinical care might be impacted." Pressed in part by faculty members whose names appear on papers alongside Poehlman's, Vermont is asking geographically dispersed co-authors whether they can vouch for the data in an additional 125 papers or so—all those published since 1995.

The investigation into Herrmann and his colleagues, who had published many studies of drug effects on cancer cells, was even more exhaustive. DFG asked Rapp to examine more than 600 publications. Herrmann was a co-author on 347 of them. "My main goal was to clear up the literature—that's a community service," says Rapp. The work with clinical implications left him especially queasy. "Just imagine someone in New Zealand reads this paper and says, 'That's cool, I can do this with my patients,' " says Rapp.

In other fields, investigators may worry less about dissecting a scientist's multiyear oeuvre. The name of Jan Hendrik Schön, a Bell Labs physicist at the center of one of the most notorious fraud cases in the physics world, appeared on more than 90 papers. After whistleblowers a *Nature* paper retracted the year before by its authors.

In 2003, immunologist Michael Croft of the La Jolla Institute for Allergy and Immunology in San Diego, California, published an article in *Nature Reviews Immunology*. Unbeknown to him, his references included a paper co-authored by Herrmann that was retracted in 1997; it had appeared in 1996 in the *Proceedings of the National Academy of Sciences* (*PNAS*). "I actually had no idea that paper had been retracted," says Croft.

Although the Internet has made it easy to link retractions to articles, "if something has been published in a paper journal and been bound, and then retracted later, no one's going to know," says *Science* Editor-in-Chief Donald Kennedy. And even online retraction notices don't always get picked up. Croft, for example, says he had overlooked the retraction notice tacked to Herrmann's *PNAS* paper online.

An online retraction notice probably wouldn't have helped biochemist Hans Vogel of the University of Calgary in Alberta. Vogel says he was "unnerved" to learn from *Science* that his 2005 *Biochemistry* article cited a paper that had been retracted from *Cell* 4 months earlier. Although Vogel's paper was submitted before *Cell* issued its retraction, he says, "I would have probably cited it again." Like many scientists, Vogel keeps records of papers he's cited in a personal electronic reference manager, and it's not updated to include retractions.

Vogel suggests that journals should play a more active role in purging the literature of retracted data. When they publish a retraction, he believes, they should alert those who previously cited the work. But journal editors say they don't have the resources to prevent retracted papers from enduring. Checking every citation in submitted papers, for example, "would be beyond our means," says Richard Dodenhoff, journals director of the American Society for Pharmacology and Experimental Therapeutics, which publishes five scientific journals. "We don't look up every reference, and I know our reviewers don't" either. Still, reviewers intimately familiar with a given field "ought to recognize a name that's suspicious" in a citation, says Friedman.

PAPER TRAIL

Field: Plant biology
Published: *Science*, 19 July 1996
Retracted: 21 May 1999
66* citations before retraction
46* citations after retraction
* Approximate.

Publicity may be among the best tools to keep the lid on retracted papers. In her 2005 master's thesis tracing retracted publications, librarian Mary Gabehart, now at Cato Research in Research Triangle Park, North Carolina, measured a gradual drop in citations after a 2000 Schön paper from *Science* was retracted in 2002. (The paper has been cited 25 times since.) In contrast, *Science* found that a 1996 paper co-authored by Inge Czaja retracted from the journal in 1999 with less publicity garnered roughly 46 citations after it was pulled (out of about 112 in all). "The less media coverage it receives," says Gabehart, "the more likely it [is] to continue to be cited."

–KATHERINE UNGER AND JENNIFER COUZIN

alerted Bell Labs in the spring of 2002 to an identical figure in two papers, Bell Labs launched an investigation. But it chose to examine only papers about which concerns were being raised, 25 in all.

"Our committee was put together to investigate allegations of scientific misconduct, not to go look for it," says Malcolm Beasley, a physicist at Stanford University in California who headed the 4-month inquiry. That arrangement was driven in part by the numbers of co-authors involved. Bell Labs was also under heavy pressure to assess Schön's fakery quickly. Beasley's committee found that 17 papers contained fake data; all were retracted, along with an additional 14 that had been based on the suspect work.

Both Beasley and Lydia Sohn, one of the Bell Labs whistleblowers who is now at the University of California, Berkeley, believe that because so many of the papers examined were fraudulent, the rest can safely be considered tainted. "The burden of proof shifts under these circumstances," says Beasley. Adds Sohn: "Pretty much people have written off" anything by Schön.

"Over some measure of time, the community … has a way of self-correcting," agrees Samuel Kaplan, a microbiologist at the University of Texas, Houston, and chair of the American Society for Microbiology (ASM) publications board. ASM journals published one paper by Herrmann, one by MIT's van Parijs, and one by Hajra, Collins's student. (The latter's work was retracted.) Referring generally to older and clearly fraudulent research and speaking for himself, Kaplan argues that "if those papers are hanging out there, they're probably not going to do any harm. We could go ahead and say in a journal in 2006, a paper published in 1997 is suspect—but no one's going to do that."

The investigation "was a waste of time."

—Ulf Rapp
University of Würzburg

Collateral damage

Between clear fraud and clean data lies a vast sea of gray. Inevitably, investigators label some papers "suspicious" but not definitively fake. University investigations fail to confirm fraud without ruling it out. Some fraud inquiries sought by worried journals never launch.

"We don't condemn people for crimes that we couldn't prove," says *PLoS*'s Cohen, pointing out the difficulties of retracting a suspect article. But at the same time, suspicions of fraud that are never established put journals in an uncomfortable position. These uncertainties are "the Achilles' heel of the process," says Kaplan, and no one seems to know how to handle them.

Legal concerns weigh heavily. "I have not seen actual litigation, but I have seen threats of it," says Debra Parrish, an attorney at Parrish Law Offices in Pittsburgh, Pennsylvania. Threats are sometimes enough to discourage journals from taking action. Dodenhoff, who oversees *Molecular Pharmacology*, recalls an instance when the journal stepped away from alerting readers to suspicious findings for fear of a lawsuit. "We're fairly small, we don't have a lot of money, and frankly we backed off," he says.

Attorneys say that journals' legal fears are sometimes overblown. "If there has been a complete due process investigation, … a journal can rely on a report," says Barbara Mishkin, a partner at Hogan & Hartson in Washington, D.C. But retractions in suspicious cases are "going to be a close call for the journal editor," she says. In theory, publications could be sued for libel or defamation, explains Mishkin, or even contract interference, if the retraction torpedoes a scientist's chance for a job for which he'd been under consideration.

Occasionally, journals take calculated risks, retracting a paper even absent misconduct findings or author unanimity. In late 2004, *Cell* and the *Proceedings of the National Academy of Sciences* (*PNAS*) retracted papers co-authored by Meena Chandok, a plant biologist who had been working at the Boyce Thompson Institute (BTI) for Plant Research in Ithaca, New York. According to a lawsuit later filed by Chandok, her supervisor, Daniel Klessig, "accused her of falsifying her research data" and requested the retractions. BTI also began conducting an investigation.

PNAS's executive editor, Diane Sullenberger, said in an e-mail message that the journal attempted to get all the authors to agree to the retraction but couldn't locate Chandok. It pulled the paper anyway. The retraction mentioned that Chandok had not signed off on it, a stance Sullenberger says the journal felt comfortable with.

In the end, BTI's president David Stern said in a memorandum, the allegation was "not substantiated," although he noted "numerous disputes on factual issues." Chandok sued Klessig last August for defamation that her lawsuit claims has "significantly damaged" her scientific reputation. The journals were not included in the suit.

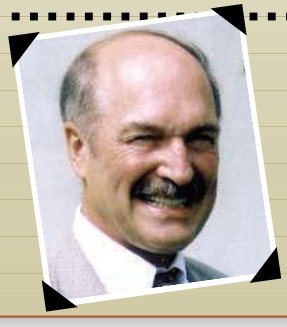
If the authors don't initiate retractions, publications usually wait for the findings of an investigation before assessing their options. Doing otherwise carries grave risks, editors believe. "Our lawyers have told us that we [would be] wide open for a lawsuit," says Kaplan.

But what happens when an investigation doesn't cover a particular journal's papers, or isn't undertaken at all? Since stem cell scientist Woo Suk Hwang, formerly of Seoul National University, was accused last fall of one of the most brazen frauds in recent memory, multiple investigations have swung into gear in South Korea and the United States. It's not yet clear how many of the dozens of papers by Hwang and his collaborators at MizMedi Hospital and Hanyang University in Seoul the investigators will be examining.

That's prompting some editors to take matters into their own hands. At *Stem Cells*, executive

CASE FILE

Name: Friedhelm Herrmann
Age: 58
Institution: Institutions in Freiburg, Berlin, and Ulm, Germany
Discipline: Oncology
Paper Trail:
Author or co-author of 347 papers
94 publications found to contain falsified or suspicious data
19 papers retracted, two corrected
Current status: Working as an oncologist in Munich



FRAUD INVESTIGATION
Began: 1997
Concluded: 2000
Run by: University-organized committee, DFG-appointed panel

Ulf R. Rapp,
Leader of the investigation

PHOTO CREDIT: S. PRÄNGER/INSTITUT FUER MEDIZINISCHE STRAHLENKUNDE UND ZELLFORSCHUNG (MSZ)

editor Martin Murphy is angst-ridden over a paper the journal published in 2004, whose nine authors include seven Hwang collaborators. Desperate to learn whether the paper is fraudulent, he has contacted officials at Seoul National University, MizMedi Hospital, Hanyang University, and the University of Pittsburgh, where two of the scientists had been working. No Korean investigation has scrutinized the paper. A Pittsburgh dean, he says, told him that "since no one was of employ at Pittsburgh at the time the papers were published, it's outside our review."

Murphy's concern is twofold: correcting the literature in his journal, and shielding the innocent. "You really have to protect those folks who were swept along," he says. Unable to identify the blameless, *Stem Cells* has for now banned articles by any of the nine authors. On 2 April, *Stem Cells* issued an editorial retraction of the paper.

Sometimes investigations simply don't happen or quickly peter out. "Papers from outside Northern Europe, Australia, North America, some countries where regulatory bodies are extremely reluctant to get involved, we usually have to give up in the end," says Harvey Marcovitch, chair of the Committee on Publication Ethics, a U.K. group that advises journals on ethics matters. Geographic patterns aside, this can happen anywhere.

In 2000, for example, the *British Medical Journal* (*BMJ*) rejected a paper by Ranjit Kumar Chandra, a nutrition researcher then at Memorial University in Newfoundland, Canada. The work examined how a multivitamin improved the memory of older individuals. But a *BMJ* reviewer had concerns about its statistical analyses, says Richard Smith, then the journal's editor. Concerned, Smith contacted Memorial.

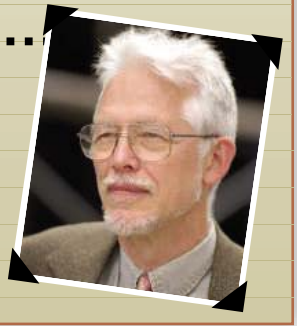
Unbeknown to him, the university had been handling complaints against Chandra since the early 1990s, according to a statement now posted on its Web site. But "repeatedly Dr. Chandra avoided fulfilling" the university's requests for his data, the statement notes, at one point claiming "that the data had been stolen." Christopher Loomis, vice president of research at Memorial, says that absent Chandra's cooperation, the school couldn't reach a definitive outcome. "We ended up with a 'he said, she said' situation," he says.

Meanwhile, in 2001 Chandra published in *Nutrition* the paper originally submitted to *BMJ*, says Smith, who contacted Michael Meguid, the journal's editor, to let him know. *Nutrition* took the rare step of launching its own investigation, says Meguid. Meguid asked outside scientists to examine the study's data, and in 2005 the paper was retracted. The *Nutrition* retraction notice described statistical concerns and added that "Chandra failed to declare that he … has a financial stake" in a vitamin supplement formula the study was testing.

CASE FILE

Name: Jan Hendrik Schön
Age: 35 or 36 (born 1970)
Institution: Bell Labs
Discipline: Physics
Paper Trail:
Author or co-author of 90+ papers
25 papers investigated
17 papers found to involve scientific misconduct
Current status: Fired from Bell Labs the day the investigation reached its conclusions. Stripped of his Ph.D. by University of Konstanz in 2004. Whereabouts unknown.

FRAUD INVESTIGATION
Began: May 2002
Concluded: September 2002
Run by: Bell Labs



Malcolm Beasley,
Leader of the investigation

Chandra has published some 200 articles in a career that dates back more than 30 years. The *Nutrition* paper was the only one for which *Science* found a retraction.

In general, in the absence of firm investigative findings or unanimous author requests for a retraction, journals do not retract a paper. Increasingly, however, they are seeking a middle ground to accommodate the fuzziness they keep encountering. The "Expression of Concern," an editorial acknowledging worries about a paper without formally pulling it, has been popping up more and more. In the Sudbø case, for example, *The New England Journal of Medicine* quickly published an expression of concern about the aneuploid papers after noting duplicate images in one, although Sudbø had not personally and publicly confessed to faking them and the investigation was just beginning.

A creative approach was taken by a senior scientist trying to clear his name after the Max Planck Institute for Plant Breeding Research in Cologne, Germany, concluded that his lab technician had falsified experiments. In 1999, plant biologist Jeff Schell of Max Planck published a paper in *The Plant Journal* detailing eight papers co-authored by the technician that he and his colleagues stated could not be replicated. Two other papers on which the technician was an author had already been retracted.

Schell's paper was handled delicately by the journal and published like any other new finding, says Irene Hames, now managing editor of *The Plant Journal*. As a condition of publication, Schell sent letters of correction to each of the journals that had run the articles in question. "I think the journal is the primary point of enforcement" against fraud, says Harry Klee, *The Plant Journal*'s current editor. "In the end, it's our process that got that work into publication."

"The journal is the primary point of enforcement" against fraud.
"In the end, it's our process that got that work into publication."
—Harry Klee, Editor, *The Plant Journal*

But that attitude doesn't seem to be widely shared. Eight years after undertaking his mammoth investigation of Herrmann, Rapp's tone is flat and dispirited. "It was a waste of time," he says now. *Science* found that just 13 of the 29 "falsification-beset" papers were retracted. Six others in the suspect category, out of 56 papers, were also pulled, and two corrected. Problematic papers identified by Rapp's investigation still litter the scientific record. Their influence on ongoing research is anyone's guess.

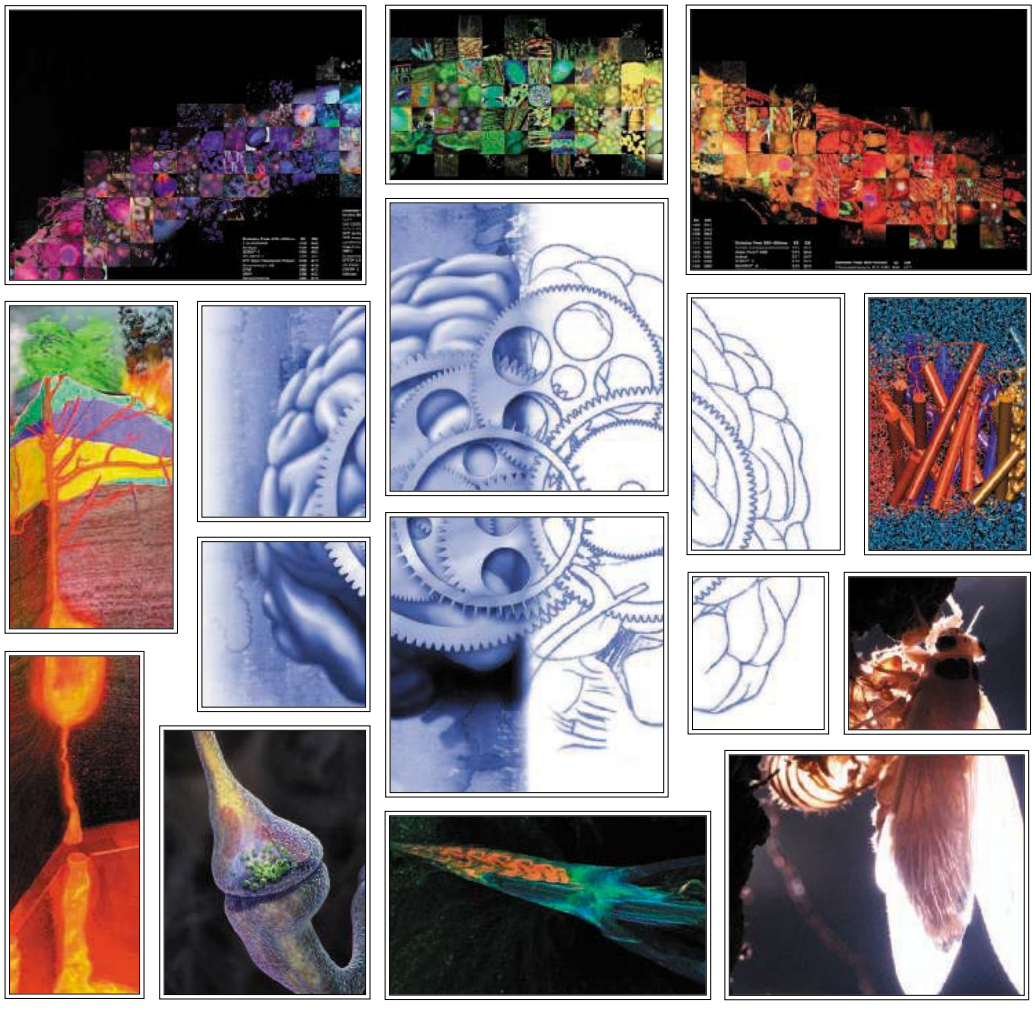
–JENNIFER COUZIN AND KATHERINE UNGER

PHOTO CREDIT: LINDA A. CICERO/STANFORD NEWS SERVICE

SCIENCE & ENGINEERING

VISUALIZATION CHALLENGE

CALL FOR ENTRIES



SCIENCE AND ENGINEERING'S MOST POWERFUL STATEMENTS ARE NOT MADE FROM WORDS ALONE

When the left brain collaborates with the right brain, science emerges with art to enhance communication and understanding of research results—illustrating concepts, depicting phenomena and drawing conclusions.

The National Science Foundation (NSF) and the journal *Science*, published by the American Association for the Advancement of Science, invite you to participate in the fourth annual Science and Engineering Visualization Challenge. The competition recognizes scientists, engineers, visualization specialists and artists for producing or commissioning innovative work in visual communication.

ENTRY DEADLINE: MAY 31, 2006

Award categories: Photographs, Illustrations, Interactive Media, Non-Interactive Media and Informational Graphics. Winners in each category will be published in the Sept. 22, 2006 issue of *Science* and *Science* Online, and will be displayed on the NSF Web site.

Complete Entry Information: www.nsf.gov/news/special-reports/scivis/index AND www.sciencemag.org/prizes/

U.S. EDUCATION POLICY

NSF Board Wades Into Swirling Debate on School Reform

A new commission hopes to point the way toward helping U.S. students do better in science and math

What ails U.S. science and mathematics education? The candidates include teachers who don't know the subject matter, lousy textbooks, a badly designed curriculum, low expectations by educators and parents, an outmoded school calendar, and the debilitating effects of poverty and race.

These causes, and many more, have been offered up over the years in innumerable reports to explain U.S. students' dismal showings on international tests. So why did a presidentially appointed oversight body for the National Science Foundation (NSF), whose responsibilities include improving science and math education at all levels, decide last week to take another bite at this well-chewed apple?

"We decided that someone needs to look at what's feasible, what's affordable, and what's politically acceptable," says Purdue University president emeritus Steven Beering. A member of the National Science Board, Beering chaired a trio of recent hearings leading up to the board's decision to form a blue-ribbon panel on improving student achievement at the elementary, secondary, and undergraduate levels (nsf.gov/nsb/edu_commission).

Agreeing that the country doesn't need "one more report on the problem," board chair Warren Washington says that he hopes the 15-member commission, to be appointed by mid-May, will instead produce an "action plan" covering needed improvements in curricula, teacher training, and evaluation. It will also describe the appropriate role for NSF and its $800-million-a-year Education and Human Resources (EHR) directorate.

Whatever they conclude, says Washington, panel members will also need to sell their advice. Federal intervention in what is traditionally the purview of local and state governments is often controversial, and the panel's recommendations could also bump up against provisions of President George W. Bush's signature No Child Left Behind (NCLB) education program. For example, NCLB penalizes schools whose students don't continue to improve on annual math and reading tests (a science test will be added in 2008) but leaves it up to states and local districts to devise standards for measuring that progress. In addition, critics say the law has been severely underfunded.

Beering, the odds-on favorite to be chair, would like the panel to tackle incendiary topics such as differential pay for science and math teachers, a year-round school calendar, and standard curricula. He's also anguished by the yawning achievement gap between schools in rich and poor neighborhoods. "We need a dialogue on these and other issues," he says. "The problem is so enormous, and it's going to take significantly more resources. At the same time, there's a lot we can do without additional investment."

School's in. Legislators heard last week about reforming K–12 science and math education from five Administration witnesses: (*from left*) Education Secretary Margaret Spellings, NSF's Arden Bement, NASA's Shannon Dale, NOAA's John Kelly, and Energy's James Decker.

CREDIT: J. DAVID SIMS/HOUSE SCIENCE COMMITTEE

There is no shortage of efforts already under way. In fact, the White House has implied that that could be part of the problem. Last month, Education Secretary Margaret Spellings convened the first meeting of a cross-agency panel examining what the Administration calculates are 207 education programs spending $2.8 billion a year to improve math and science education, half of which have budgets less than $1 million. The group will compile an inventory on the way to determining what programs are effective and, thus, deserving of continued support. At the same time, her department is poised to begin a review of precollege math curricula and teaching with the hope of funneling $250 million next year into elementary and middle schools that use high-quality materials and proven training methods.

Coincidentally, last week also featured a first-ever gathering before the House Science Committee of Spellings, NSF Director Arden Bement, and officials from three other science agencies—NASA, the National Oceanic and Atmospheric Administration (NOAA), and the Department of Energy. The quintet discussed efforts to improve precollege science and math education. Not surprisingly, the Administration officials and legislators agreed more on the severity of the problem than on how to solve it.

One point of contention is the educational component of Bush's proposed American Competitiveness Initiative (*Science*, 17 February, p. 929). Spellings defended the decision in Bush's 2007 budget request to give her department the entire $390 million. Two good ways to improve the quality of secondary school teaching, she added, would be to prepare more people to teach advanced-placement courses and to recruit scientists and engineers from high-tech companies and the military.

But both the committee chair, Representative Sherwood Boehlert (R–NY), and ranking minority member, Representative Bart Gordon (D–TN), took the Administration to task for not giving NSF a major role. In addition, Gordon questioned why 70% of the initiative would be spent on elementary and middle school math materials when there are so many other areas of great need. In particular, Gordon cited a warning to the panel last month from 2001 Nobelist Carl Wieman, who has focused on improving undergraduate physics education, that "unless you improve science education at the college level, you are wasting your time and money on trying to make major improvements in K–12."

These and other issues are exactly why Boehlert had urged the science board to keep its focus on NSF. "We're afraid that they will give short shrift to [reviewing] the adequacy of NSF's programs," says David Goldston, the House committee's chief of staff. "Given the anemic state of EHR's budget, there's a real question of whether NSF's programs will grow or wither away. And if NSF gets caught up in a controversy surrounding one of the panel's recommendations, then so much the worse."

Washington says that it's impossible to separate NSF's role from the larger issues. He also believes that the panel needs to take a national view to influence legislators and the White House. He hopes the panel will issue a report within a year of its first meeting.

–JEFFREY MERVIS

ARAB SCIENCE

Qatar Taps Wells of Knowledge

This small Persian Gulf emirate is preparing for life after oil and gas by pouring wealth into education and research

DOHA, QATAR—On this sun-scorched campus, the range of architectural styles is extreme: from futuristic rectangles surrounding a giant egg to a more traditional earth-toned arabesque building. Prominent signs indicate the presence of educational heavyweights, including Weill Cornell, Carnegie Mellon, Georgetown, and Texas A&M. From the pale, sandy ground and barren landscape, this could be any number of places in the southwestern United States. But it isn't. This unusual gathering of American academic muscle is on the shores of the Persian Gulf.

Welcome to Education City. This 1000-hectare international campus outside Doha is the centerpiece of Qatar's ambition to become the brainy hub of the Gulf region. The small emirate—home to just 800,000—is sitting atop the world's third largest natural gas reserve, and while the money keeps flowing, it hopes to reinvent itself as a knowledge economy. "What we have here is unique," says Egyptian-born Ahmed Zewail, a professor at the California Institute of Technology in Pasadena and 1999 chemistry Nobelist who is advising the efforts in Qatar. "They are overhauling the educational system from bottom to top," not to copy the West, he says, but "to find a model suited to the country."

Qatar is not the only Gulf state to realize that one day the oil will run out and that education may be the key to prosperity when it does. The emir of Sharjah, part of the United Arab Emirates, recently injected millions of dollars into local universities and tried to create a research foundation (*Science*, 5 December 2003, p. 1652). And just weeks ago, Dubai announced the opening of a branch campus of Canada's University of New Brunswick. However, Qatar is aiming to reform education from first grade to Ph.D. level, and with spending in the billions, its efforts dwarf other initiatives in dollar terms. Lars Erslev Andersen, a Middle East scholar at the University of Southern Denmark in Odense, characterizes Qatar's efforts as "the region's most ambitious, far-reaching, and focused."

It's not just the money that makes world-class institutions set up shop so far from home but an urge to spread American academic values. "This has never been done [by a U.S. medical school]," says Daniel Alonso, dean of the Weill Cornell Medical College in Qatar. "Bringing our standards and degrees abroad is challenging, and by coming to the Gulf, we engage in what I'd call educational diplomacy."

Cultural revolution

To transform Qatar is the grand vision of its emir, Hammad bin Khalifa Al-Thani. After seizing power from his father in a bloodless coup in 1995, Sheikh Hammad began a modernization drive that included the creation of the independent Qatar Foundation for Education, Science, and Community Development, with an endowment reputed to be in the billions of dollars. It is run by the emir's wife, Sheikha Mozah bint Nasser Al-Misnad.

One of the foundation's chief beneficiaries, Education City, is a work in progress. Three years after the official launch, three-quarters of the planned buildings are still taking shape amid a forest of cranes. In its final incarnation, the now-dusty campus will boast green lawns, palm trees, student housing, cafés, and shops. The foundation has attracted some top-flight U.S. universities to set up shop here by offering to pay for all buildings, overheads, and staff salaries. Student fees go straight into the universities' coffers back home. Today, there are only 500 students at Education City, 60% Qatari nationals and the rest recruited mainly from Arab countries. The plan is to enroll a broad international crowd of 8000.

Qatar is "undergoing a cultural revolution," says geologist Saif Al-Hajari, vice chair of Qatar Foundation. "We cannot live in a closed world but must invite a mixture of cultures, nationalities, and ideas." Mohammad Hassan, director of the Third World Academy of Sciences in Trieste, Italy, agrees: "Embracing globalization and emphasizing quality in education is critical to development in the Arab world but so far lacking." If Qatar's experience is positive, he thinks the initiatives will inspire governments around the region.

The revolution begins in Qatar's primary and secondary schools, which have begun to dispense with traditional rote learning, Al-Hajari reports, replacing it with curricula designed to stimulate creative and independent thinking. And it extends to Qatar University, which was founded in 1977 and is independent of Education City. In 2003, the emir appointed Sheikha Abdulla Al-Misnad, an educationalist trained at the University of Durham in the U.K., as president. Her mandate is to turn what she calls a "typical Third World institution" into a modern and competitive university.

According to Al-Misnad, wealthy Gulf societies have fostered a "culture of entitlement" among young people: "Students feel they have a right to a degree whether they qualify for university or not." Al-Misnad has set about upgrading the university's six colleges to qualify for international accreditation and has applied quality control and accountability to all levels of the institution.

Women to the fore

This overhaul of Qatar University has had an unexpected side effect: Three-quarters of the students accepted for admission are now

CREDIT: MARTIN MARION

Oasis. With the help of government grants, Western universities have raised their tents in Qatar's desert.

female. Although this may seem a triumph in a region where higher education is often out of bounds for women, Al-Misnad sees a potential backlash if future generations of men are not as well educated as women. She has launched a study into how young and restless Qatari men can be motivated to take up higher learning. It may not be easy, suggests one student, who asked to remain anonymous, from Georgetown University's outpost in Qatar. "After high school, most young men here just want to have fun and perhaps get a job to be independent. At 16, the girls are not allowed to do that. Their only option is to study," he says.

Because many girls are not allowed to go abroad alone to study, their best option for a first-class international education is Education City. Suresh Tate, who teaches biochemistry and basic science at Weill Cornell here, has been struck by the "tremendous enthusiasm and commitment of women students." And Dean Charles Thorpe at Carnegie Mellon's facility reports that "academically, the women generally outdo the men." According to 18-year-old Noor Al-Maadeed, who studies computer science at Carnegie Mellon: "New opportunities are opening up for women, and personally I want to work to help my country develop further."

Although students may be thriving academically, adapting to the ethos of American institutions has not been without problems. These are Qatar's first co-ed institutions, and this causes friction, as when students at the Texas A&M outpost recently asked for gender-segregated lounges. Dean Mike Kemp urged them to look over the horizon: "My answer was: We are trying to make them into engineers. Out there in the workplace, they can't control whether their colleagues are men or women."

Qataris have criticized Education City at times in the op-ed and letters pages of local newspapers. "Some believe we should develop local universities rather than import foreign ones," says Abdulla Al-Thani, the Qatar Foundation's vice president for academic affairs. "This is a major cultural experiment, and changes toward more westernized norms cannot happen overnight," says Texas A&M technical consultant Naguib Ktiri-Idrissi, adding that some students have told him that more conservative families are reluctant to send their children to Education City. Despite this, says economist Ibrahim Oweiss of the Georgetown facility, the modernizers are advancing. "The reforms taking place are radical and happening at a fast pace," he says, but the "immense coherence" of Qatari society will enable it to absorb change.

Icing on the cake

In spite of the reservations, Education City is set to grow. The Qatar Foundation wants to add as many as four more branch campuses before the end of the decade and is holding talks with universities in Europe and Asia to provide selected programs. Beyond that, a leap into postgraduate study and genuine research is being planned. Abdulla Al-Thani regards this as "necessary to make the education drive sustainable and to develop home-grown knowledge industries."

Arab countries do not, however, have a good track record in science, spending just 0.15% of gross domestic product—little more than a 10th of the global average—on research, according to United Nations figures. Qatar aims to buck the trend. Education City is equipped with teaching and lab facilities that would be the envy of most Western universities. At the branch campuses, research by faculty members is funded by the Qatar Foundation. The foundation will also launch a grant-giving fund this year, under the watchful eye of international peer review. In another initiative, Skeikha Mozah has dedicated the proceeds from an entire oil well—as much as $80 million per year—to research, creating a fund known as the Well of Knowledge.

Research programs at the branch campuses have so far tended to align with Qatar's needs. Texas A&M is setting up joint research with the oil industry and studies related to clean air, while Weill Cornell will concentrate on biomedical projects relevant to local health problems. "With the world's third highest diabetes incidence, Qatar is well placed for diabetes research," says Alonso. Further down the line he also has an eye on embryonic stem cell research—a no-go in New York but legal in Qatar.

To accelerate the process, Alonso plans to bring interested scientists from Weill Cornell's New York base and recruit postdocs. For his part, Al-Thani hopes to lure back expatriate Arab scientists currently flourishing in the West. "Many diaspora researchers are interested in going back if the infrastructure is there," says Hassan. To capitalize on the research, the Qatar Foundation is building the Qatar Science and Technology Park right next door to Education City. Already, big players in industrial R&D, including GE, Microsoft, and ExxonMobil, have signed up and are waiting to move in.

But could Qatar—a country without the slightest research tradition—become the Singapore of the Arab world? "It's a possibility," says Zewail. "But it's important to realize that great buildings and big budgets are not enough; the essential thing is a system that appreciates scientists."

–LONE FRANK

Lone Frank is a science writer and author in Copenhagen.

Across cultures. Cornell University's Weill Medical College runs training programs in diabetes and others areas of local concern, and aims to lay the foundation for research careers.

CREDIT: MARTIN MARION

ATMOSPHERIC SCIENCE

Technique From Outer Space Takes On Earth Observation

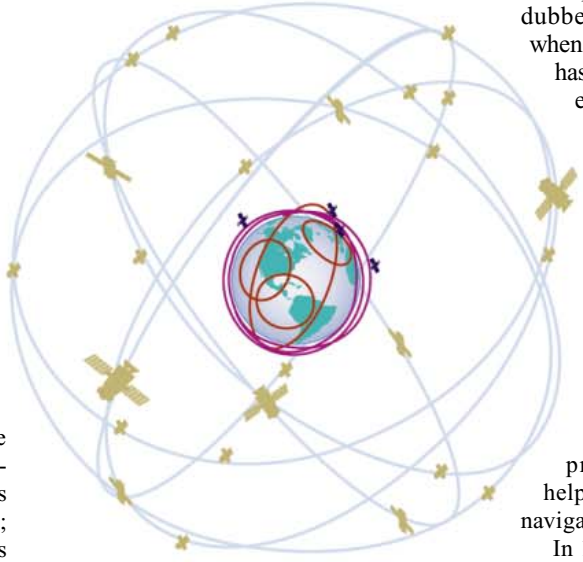
By keeping a close eye on GPS satellites, a team of researchers hopes to measure atmospheric temperatures on the cheap

Weather forecasting has come a long way since the era of dog-eared almanacs and barn-door barometers. Nowadays, numerical models running on the world's most powerful supercomputers crunch data from a host of sources that include ground-based weather stations, radar, aircraft, and satellites. But one key type of information—vertical profiles of how temperature, pressure, and water vapor vary through the atmosphere—is still gathered the same way it has been since the 1930s: with weather balloons.

Locked on. Cosmic satellites (blue, *right*) track signals from GPS satellites (beige, *right*) and log how the atmosphere bends them (*above*)—data that reveal temperatures.

Every day at noon and midnight, hundreds of them rise from stations around the globe bearing small instrument packages skyward. Each "radiosonde" beams down data as it climbs to an altitude of about 30 kilometers; then the balloon bursts, and the instruments parachute back to Earth. The system isn't perfect. The balloons fly only twice a day and are concentrated over the populated Northern Hemisphere. The oceans and the south are data deserts. It's wasteful, too. The U.S. National Weather Service estimates that of the 75,000 radiosondes it launches every year, 80% disappear without a trace. So far, however, no other data-gathering method can match their accuracy and vertical resolution. "Radiosondes are the gold standard," says atmospheric physicist Sean Healy of the European Centre for Medium-Range Weather Forecasts (ECMWF) in Reading, U.K.

That may be about to change, thanks to six simple satellites collectively known as Cosmic, due for launch in mid-April. Each carries a Global Positioning System (GPS) receiver, a communications link to the ground, and not much else. The receivers will lock onto transmissions from the U.S. Air Force's constellation of 24 GPS satellites and watch how the atmosphere bends the radio waves. From that information, each Cosmic spacecraft can get a vertical profile of the atmosphere's temperature, pressure, and water content above one spot on Earth's surface with surprising accuracy and vertical resolution. All together, the fleet will make 3000 "soundings" a day, evenly distributed across the globe.



Cosmic is an academic research project largely financed by the Taiwanese space agency, but its proponents expect it to have a major impact on day-to-day weather forecasts: Meteorologists at the world's major weather agencies are poised to receive Cosmic's data. "It will be a major breakthrough in the science of climate, … [providing] a whole new scale of accuracy," says atmospheric chemist James Anderson of Harvard University. Anderson likens current efforts to measure the state of the climate to trying to reconstruct a rugby game from five or six fuzzy photographs. Cosmic data will be like having "thousands of high-resolution pictures" of the match, he says.

For atmospheric scientists used to large, expensive Earth-observation satellites operated by the likes of the U.S. National Oceanic and Atmospheric Administration and EUMETSAT, Europe's weather satellite operator, GPS sounding is an unknown quantity. "For most, it is still a strange idea from out in the wilderness," says sounding pioneer Thomas Yunck of NASA's Jet Propulsion Laboratory (JPL) in Pasadena, California. But he predicts Cosmic data will be "far beyond anything we have imagined" and will lead to a "major sea change in atmospheric sensing." Healy agrees: "When people see the data and the quality of it, they will be won over."

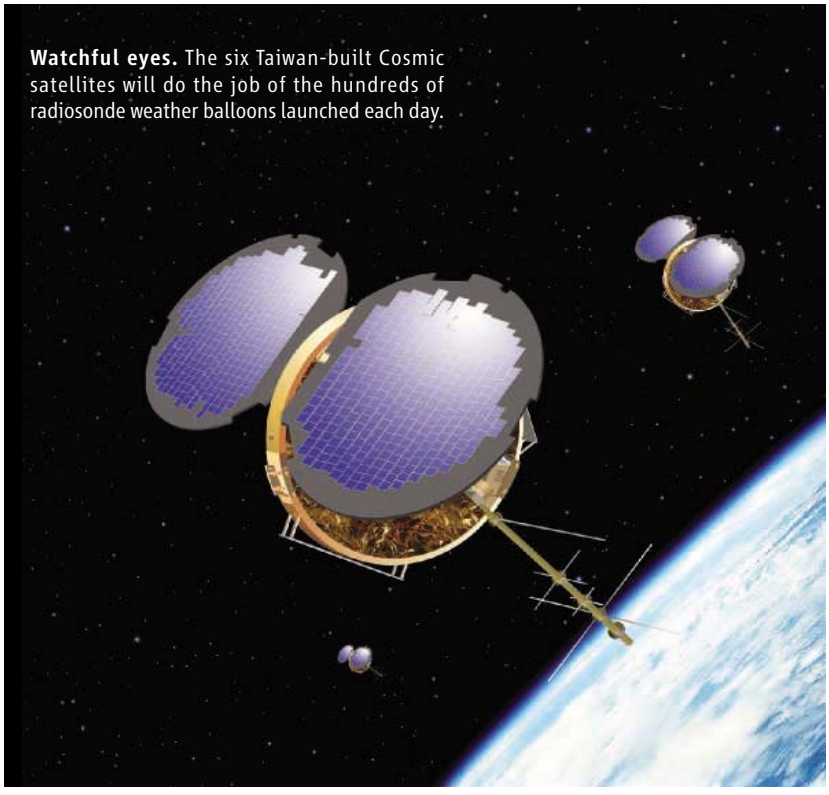
From outer space

The methods that Cosmic will use were first forged deep in space. In the early days of planetary exploration, researchers realized that as a spacecraft passed behind a planet during a flyby, the planet's atmosphere would briefly refract radio signals passing through it en route to Earth. In the early 1960s, teams at JPL and Stanford University worked out how to use the radio signals to deduce information about planetary atmospheres. The technique, dubbed radio occultation, made its debut when Mariner 4 visited Mars in 1965, and it has now probed the atmosphere of almost every planet in the solar system and many of their moons.

Closer to home, however, radio occultation had a harder time finding a niche. Researchers weren't sure that the new technique would be more accurate than established methods for studying the atmosphere. What's more, to achieve global coverage, an occultation-based system would need a constellation of satellites transmitting radio signals—an unthinkably expensive proposition until the U.S. Air Force helpfully began lofting GPS satellites for navigation in the 1980s.

In 1988, a team at JPL, including Yunck, won approval for a proposal to put GPS receivers for radio occultation onto all the craft of NASA's Earth Observing System and other satellites, but the plan fell victim to budget cuts. Researchers at the University Corporation for Atmospheric Research (UCAR) in Boulder, Colorado, had better luck with a National Science Foundation–funded project to launch a proof-of-principle receiver. The GPS/MET instrument took to the skies in 1995 aboard NASA's Microlab I satellite; it exceeded everyone's expectations. The experiment produced data for 2 years, and UCAR researchers achieved vertical resolution of 100 meters and temperature accuracy of better than a degree. "GPS/MET was a great success," says Bill Kuo of UCAR, director of the

CREDITS: ADAPTED FROM COSMIC/UCAR



Watchful eyes. The six Taiwan-built Cosmic satellites will do the job of the hundreds of radiosonde weather balloons launched each day.

CREDITS (LEFT TO RIGHT): COSMIC/UCAR; UNIVERSITY OF ALASKA FAIRBANKS

Cosmic project. NASA then sponsored receivers to fly on the Danish Ørsted mission, South Africa's Sunsat, Argentina's SAC-C, and Germany's CHAMP.

CHAMP, which has operated in low Earth orbit continuously since 2000, gave researchers a chance to hone occultation into a precision instrument. To take a sounding, a GPS receiver on the satellite locks onto the signal from a GPS satellite descending toward the horizon. As it gets lower, the atmosphere bends the path of the signal and delays its progress, until the limb of Earth cuts it off altogether. The whole process takes between 1 and 2 minutes.

CHAMP ignores the navigation information that the signal carries and just looks at the underlying radio wave. "The receiver counts cycles, looking for a Doppler shift in the frequency," says JPL's Ian Harris, who worked on the Cosmic receivers. The shifts give the bending angles; from the angles, researchers can calculate the atmosphere's refractivity at each altitude. A computer model of the atmosphere can then work out a profile of temperature and water vapor from the refractivity. "There is real art and elaborate theory in extracting the data," says Yunck.

Despite the evidence that GPS sounding worked and produced valuable data, researchers found it hard to get backing for a constellation of craft to produce enough regular soundings for weather forecasters. "We tried to talk to U.S. agencies, but they were focused on big, established missions," says Kuo. Many atmospheric scientists work for decades on large missions that cost $400 million apiece. A science-grade GPS receiver hardened for space, by contrast, costs only a few hundreds of thousands of dollars, and the whole of Cosmic costs $100 million. "Cheap sounding missions are potentially a threat, so the community is suspicious," says Kuo.

Kuo, who was born in Taiwan, discovered through his contacts there that Taiwan's fledgling National Space Organization was looking for projects to help build up the country's space industry. In 1997, a deal was signed in which Taiwan would assemble the Cosmic satellites with JPL-designed receivers, and UCAR would provide the ground processing and archiving. Taiwan is footing 80% of the bill.

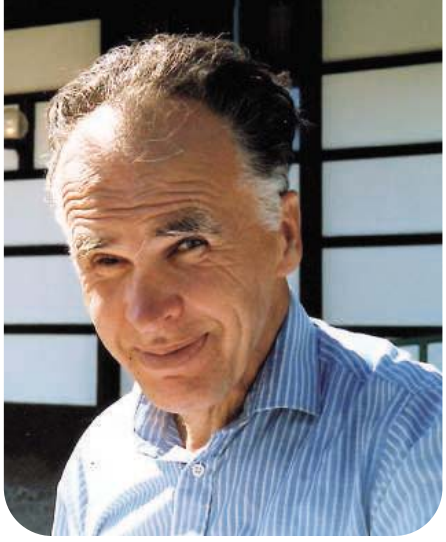
With funding from various U.S. agencies, UCAR has worked hard to streamline the processing so that the data can reach forecasters as soon as possible. With ground stations in Fairbanks, Alaska, and Kiruna, Sweden, each of the satellites can download data once per 100-minute orbit. Transfer to Boulder takes 5 minutes, and processing takes 10 to 15 minutes, so Kuo predicts that data can be in the hands of weather agencies on average 90 minutes after the sounding was made. The U.S. National Centers for Environmental Prediction, ECMWF, and the U.K. Met Office hope to start receiving the data a couple of months after Cosmic is launched. "Researchers love this stuff and are well prepared for the data," says Yunck.

Climatologists, too, are looking to GPS sounding for a solution to a long-standing problem in their field: getting consistent measures of the atmosphere. Over years and decades, instruments drift away from calibration, and new instruments may be biased differently from the ones they replace. Cosmic data don't have those problems, says Harvard's Anderson, because the frequencies the receivers record are basic measurements. "There are no fudge factors, no conversions," Anderson says. "These very high accuracy global measures produce a climate record of Earth that is permanent and unequivocal."

If GPS sounding enthusiasts are right, soon the weather will hold fewer surprises, and climate researchers will be building up an accurate three-dimensional map of the atmosphere that will keep them busy for decades. Already, some are looking ahead to an expanded "Cosmic 2" constellation or to new efforts as other agencies jump on the bandwagon. "The success of Cosmic could stimulate a multisatellite mission in Europe," says Jens Wickert of the CHAMP team at Germany's GFZ geosciences research center in Potsdam. In a possible sign of what's to come, EUMETSAT is putting a single GPS receiver on its Metop-2 satellite, due for launch in June. This will be the first truly operational radio-occultation receiver.

Before fleets of other sounding spacecraft can take wing, however, Cosmic will have to show what can be done with a few simple satellites and some GPS receivers. JPL physicist James Zumberge, for one, is betting on the underdog. "Radio occultation has a bright future," he says, "and Cosmic is the next step in that future."

–DANIEL CLERY



Awards

ADD IT UP. Lennart Carleson of Sweden's Royal Institute of Technology in Stockholm is this year's recipient of the $920,000 Abel Prize in mathematics from the Norwegian Academy of Science and Letters.

Carleson, 78, is best known for a 1966 paper proving a long-standing conjecture about the Fourier series, a mathematical tool used to analyze oscillatory phenomena, from pulsars to sound waves to atomic vibrations. In 1991, Carleson and colleague Michael Benedicks gave a rigorous proof that a dynamical system known as the Henon map possesses a signature of chaos known as a strange attractor. "The remarkable aspect of Carleson's style is that his methods are very constructive—almost like algorithms—but simultaneously very ingenious and deeply original," says Benedicks.

DEATHS

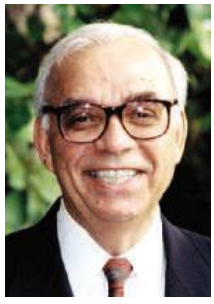
ENDURING PRINCIPLES. Nearly every student of neuroscience is familiar with Columbia University neurobiologist James Schwartz through his 1414-page textbook. Colleagues say Schwartz was working on the fifth edition of *Principles of Neural Science* up to a few days before his death from leukemia 13 March in New York City. He was 73.

Schwartz was known not only for the seminal textbook but also for his research. He advanced our understanding of the biological basis of memory, says Columbia's Eric Kandel, 2000 Nobelist and co-editor of the textbook's first edition.

Schwartz also was actively involved with the American Numismatic Society. "He was a wonderful, generous, and very cultured person with a deep interest in all of science," says Kandel.

VIRTUOSO EDITOR. Nicholas Cozzarelli, a molecular biologist at the University of California, Berkeley, and editor-in-chief of the *Proceedings of the National Academy of Sciences* (*PNAS*), died 19 March from complications related to cancer. He was 67.

The son of an immigrant shoemaker who rued his own lack of education, Cozzarelli did his dad proud by studying at Princeton, Yale, and Harvard. "I'd consider Nick a virtuoso in the field of DNA topology," says Stephen Benkovic of Pennsylvania State University in State College, of his work on how enzymes control the shape of DNA.



As *PNAS* editor since 1995, Cozzarelli promoted open access and allowed researchers to submit articles directly rather than having them put forward only by academy members. "It was one innovation after another that raised the importance and visibility of the *Proceedings*," says Richard Losick of Harvard University.

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MOVERS

CATCH THE WAVE. Scientific jack-of-many-trades Jay Marx has been named executive director of the Laser Interferometer Gravitational-Wave Observatory (LIGO), a $300 million collaboration between Caltech and MIT to detect tiny ripples in space and time. He replaces Caltech's Barry Barish, who left to lead the International Linear Collider project.

Trained as a particle physicist, Marx, 60, has made a career of leading large projects in various fields at the Department of Energy's (DOE's) national labs. "We looked around the world for an experienced leader who could take on a job of this complexity," says Peter Saulson, a physicist at Syracuse University in New York and a LIGO collaborator. LIGO has twin facilities in Washington state and Louisiana that began taking data in earnest last year and is seeking approval for an upgrade in 2008.

Marx, who retired from DOE's Lawrence Berkeley National Laboratory earlier this year, says he's eager to delve into yet another field. "This idea of looking out into the cosmos with new eyes is really exciting."

Three Q's >>

Adel Mahmoud, 64, has taken on the daunting challenge of organizing the scientific community to speed the development of an AIDS vaccine. In September, the former president of Merck Vaccines becomes the first chief executive of the Global HIV Vaccine Enterprise (*Science*, 27 June 2003, p. 2036).

Q: People have difficulty understanding what the enterprise is. What do you say to them?
We have tried investigator-initiated grants. We have tried program projects. We have tried the industrial approach. The fact is simple: 22 years, and we have no vaccine. So, can we model ourselves around a global effort in which the scientific community agrees that these are the targets we want to pursue and leaves the funders to fund pieces according to their wishes? My job is to try to apply business planning and a disciplined approach to that vision.

Q: Unlike at Merck, you can't hire people, set budgets, or apportion lab space. What actual influence can you have here?
I can use persuasion and pressure by the global community and the imperative of getting us an HIV vaccine. These are very, very credible forces.

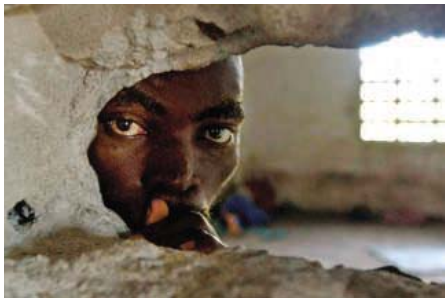
Q: Pharmaceutical companies are terrifically secretive. Will you post timelines on the Web?
Yes. If I'm going to put this on a piece of paper hidden in somebody's desk, what value would that have?

CREDITS (TOP TO BOTTOM): THE NORWEGIAN ACADEMY OF SCIENCE AND LETTERS; UC BERKELEY; CHRISTINE CELATA; JONATHAN SMITH, COLUMBIA UNIVERSITY MEDICAL CENTER; MERCK

LETTERS

edited by Etta Kavanagh

The Burden of Brain Disorders



CREDIT: JEHAD NGA/CORBIS

WE WERE THRILLED TO READ G. MILLER'S NEWS STORIES ON "MENTAL HEALTH IN DEVELOPING countries" (News Focus, 27 Jan., p. 458). He highlights a major and much-neglected component of the world's health problems. It is important to recognize that mental health disorders are but one manifestation of the many brain disorders that are overrepresented in the developing world. Cerebral palsy is 5 to 10 times more common in poorer countries. Most of the 60 to 80 million individuals in developing countries with epilepsy, many of whom are children, go untreated, despite the availability of low-cost and effective medications. Stroke is the leading cause of death in many countries, such as Ecuador, whereas prevention programs have significantly reduced their occurrence in many wealthy countries. Taken as a whole, disorders of the nervous system account for at least 15% of the global burden of disease and at least 27% of average years lived with disability (*1*).

These numbers refer only to disorders that arise within the brain. If we add the impact of the many conditions that damage the brain as part of their overall effect, such as malnutrition, trauma, infections such as HIV/AIDS, cerebral malaria and other parasites, and meningitis, the numbers become much larger, approaching 30% of the global burden of disease (*2*). In many respects, brain health and disease may be the best overall indicator of a nation's success in promoting health.

The underlying issues that lead to the increased frequency and impact of brain disorders in developing countries are very much a part of the conditions that lead to overall ill health. However, the lack of understanding and outright stigma that these disorders so often engender add yet another barrier to reducing these problems.

MARCELO CRUZ,[1,2] RACHEL JENKINS,[1,3] DONALD SILBERBERG[1,4]*

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Evolution of Metazoa and Fungi

IN THEIR RESEARCH ARTICLE "ANIMAL EVOLUTION and the molecular signature of radiations compressed in time" (23 Dec. 2005, p. 1933), A. Rokas *et al*. present apparent differences in phylogenetic resolution within the animal and fungal kingdoms, using the same set of genes. They use these discrepancies to make their point that the poor resolution among basal lineages within the metazoa is due not to mutational saturation but to a rapid radiation of animal phyla. However, the biased taxon sample of the fungal sequences does not allow this conclusion.

All fungi used were from the phyla Basidiomycota and Ascomycota, which contain the great majority of extant fungal species and are relatively recent lineages. None of the three basal and putatively more ancient fungal phyla (Chytridiomycota, Zygomycota, and Glomeromycota) are represented here, most likely due to the limited availability of genome sequences.

As the authors demonstrate, phylogenetic resolution is inversely correlated with the length of time elapsed since the cladogenetic events. Therefore, it is not surprising that the relatively "young" lineages of fungi represented here are better resolved than the more ancient metazoan lineages. A closer look at the fungal taxa reveals that some of them are even rather closely related to each other; in particular, there are two species from the same genus, *Saccharomyces*.

Thus, the higher proportion of resolved nodes in the fungal part of the tree and its higher "stemminess" are not valid arguments for a lack of mutational saturation in the metazoan lineages and a rapid radiation of early animals. If basal fungal phyla were included, the same apparent lack of resolution might be revealed in this kingdom.

DIRK REDECKER

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Response

REDECKER QUESTIONS THE VALIDITY OF THE comparison between Fungi and Metazoa reported in our recent Research Article, based on our sampling of fungal taxa. Three independent metrics support the validity of the comparison: (i) the fossil record, (ii) the consistent relative age of the kingdoms inferred from molecular clock studies, and (iii) the similar level of molecular divergence in the data sets. We consider each of the three metrics in turn.

(i) The oldest accepted fungal fossils are 550 to 635 million years old (*1*). The oldest accepted fossils of metazoan embryos are found in strata 550 to 590 million years old (*2*). These are likely representatives of "higher" Fungi (i.e., Basidiomycota and Ascomycota) (*1*).

(ii) We examined studies that simultaneously calculated dates of origin for Fungi (Basidiomycota and Ascomycota) and Metazoa (*3*–*5*). (Because molecular clock dating techniques show wide margins of error, we refrained from performing molecular clock analyses on these data.) We specifically focused on whether the date of origin of Fungi broadly overlapped with the date of origin of Metazoa, irrespective of what the absolute date estimates might have been. Although these clock-derived estimates vary widely across studies and invariably are older than the fossil-based estimates, fungal date estimates nicely overlap with the metazoan estimates.

(iii) Independent of the issue of time of origin, the two data sets are comparable: They contain essentially the same genes, with a similar number of variable sites and similar levels of molecular divergence. Yet, one data set yields a highly resolved tree, whereas the other does not. If we compared two clades that were known to be the same age but with vastly different degrees of molecular divergence, it would be no surprise that resolution differed.

In summary, all three metrics indicate that Redecker's suggestion that we compared "young" Fungi with "ancient" Metazoa is not supported by evidence. Additional sampling of more "ancient" fungal representatives would actually mean that we would compare two clades with different times of origin, thus guaranteeing the invalidity of the comparison.

The comparison of Metazoa with Fungi tells us that the amount of data we had for Metazoa was potentially adequate to resolve relationships among them—but it didn't. Redecker argues that if we had sampled additional fungal lineages, we might have found some lack of resolution. Although this may be shown in the future to be true, we think that the same argument could be made for Metazoa; had we sampled representatives from the main classes of arthropods, we would perhaps have found further lack of resolution.

Finally, it is important to note that our conclusion that early animal evolution is best viewed as a radiation compressed in time is not solely based on the comparison with Fungi but on several points of evidence.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Evaluating Education Effectiveness

I WOULD LIKE TO CLEAR UP A MISCONCEPTION that readers might derive from your News Focus story "Is the education directorate headed for a failing grade?" by J. Mervis (24 Feb., p. 1092). In the story, I am quoted as saying, "Maybe NSF [National Science Foundation] education programs need to be rethought." In fact, I believe that NSF education programs have been instrumental in creating a series of outstanding curricula for school science in the United States and have served an important role in making both scientists and schools aware of the urgent need to rethink what we mean by "science education" (*1*).

In my conversation with your reporter, I stressed the need to create a robust body of high-quality research on science education in our schools. In this context, I suggested that the NSF rethink its requirement for formal project evaluations, by which I meant that it support a broad range of methodologies aimed at producing useful and transferable knowledge. I suggested, for example, greater attention to multisite research that is designed to discover what does and does not work, and why. I also questioned an NSF tradition of discontinuing even the best programs after 5 years, with the

expectation that school districts (or others) will be able to cover the expense of continuing the programs thereafter.

BRUCE ALBERTS

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THE NEWS STORY "IS THE EDUCATION DIRECTORate headed for a failing grade?" by J. Mervis (24 Feb., p. 1092) confounds a number of important issues pertaining to the National Research Council's 2004 study "On Evaluating Curricular Effectiveness: Judging the Quality of K–12 Mathematics Evaluations," chaired by Professor Confrey. First, the story blurred an important distinction between quality of evaluations and quality of curricula: We did not say that none of the curricula are effective or that none of the evaluations had merit. Rather, we found evaluations that ranged in quality from highly flawed to very imaginative, but that taken together could not support broad claims—positive or negative—about the quality of the curricula. Second, the story failed to note that many of the studies we reviewed were not evaluations but progress reports, descriptive summaries, and advocacy pieces; that they included evaluations of commercially developed as well as NSF-sponsored curricula; and that the committee did not directly examine the 19 curricula in question. Third, the article misstated Professor Confrey's views about the importance of sustained and significant federal investment in curriculum evaluation. Contrary to the story's suggestion that past investments yielded little knowledge, her point was that they have informed us about what to avoid, about how to design rigorous evaluations, and about the need for greater attention to judging effectiveness through multiple evaluation methods.

MICHAEL J. FEUER[1] AND JERE CONFREY[2]

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TECHNICAL COMMENT ABSTRACTS

Comment on "PDK1 Nucleates T Cell Receptor–Induced Signaling Complex for NF-κB Activation"

Thomas Gruber, Michael Freeley, Nikolaus Thuille, Isabelle Heit, Stephen Shaw, Aideen Long, Gottfried Baier

We observe that protein kinase C θ (PKCθ) is phosphorylated on the activation loop at threonine 538 (Thr-538) before T cell activation. Our results are inconsistent with the conclusions of Lee *et al*. (Reports, 1 April 2005, p. 114) that the Thr-538 phosphorylation of PKCθ is regulated by T cell receptor activation. Other mechanisms, such as autophosphorylation of Thr-219, might orchestrate the cellular function of PKCθ in T cells.

Full text at www.sciencemag.org/cgi/content/full/312/5770/55a

Response to Comment on "PDK1 Nucleates T Cell Receptor–Induced Signaling Complex for NF-κB Activation"

Ki-young Lee, Jae-Hyuck Shim, Matthew S. Hayden, Jan-Schulze Luehrmann, Sankar Ghosh

In their comment, Gruber *et al*. report constitutive phosphorylation of protein kinase C θ at threonine residue 538. However, they fail to note that, consistent with our results, a number of other groups have previously reported inducible phosphorylation of Thr-538 in T cells. Although the physiological relevance of this discrepancy is unclear, substantial differences in experimental conditions and reagents may account for the conflicting observations.

Full text at www.sciencemag.org/cgi/content/full/312/5770/55b

ECOLOGY

The Dark Side of Night Lighting

David Hill

The aura of light that hangs over a city on an otherwise dark night brings into sharp focus the impact *Homo sapiens* is having on Earth. A satellite view of the planet at night reveals swathes and pimples of light, clearly identifying hot spots of human activity—Europe, the United States, India, and Japan. The light is a sign of our species extending its influence, packing action into every hour of the day and night.

Ecological Consequences of Artificial Night Lighting

Catherine Rich and Travis Longcore, Eds.

Island, Washington, DC, 2005. 478 pp. $65, £46.50. ISBN 1-55963-128-7. Paper, $29.95, £21.50. ISBN 1-55963-129-5.

"Artificial night lighting" seems a rather passive description for something that, as we can easily perceive, has such a pervasive effect on our fellow species. Based on expert reviews of the responses of a wide range of organisms, *Ecological Consequences of Artificial Night Lighting* offers a unique insight into how these effects manifest themselves. The volume's six sections cover mammals, birds, reptiles and amphibians, fishes, invertebrates, and plants. Each begins with a vignette on ecology at night, either excerpted from earlier accounts (e.g., writings by Alexander von Humboldt and Henry David Thoreau) or experiences described for the book (e.g., short essays by Bernd Heinrich and Carl Safina). The editors, Catherine Rich and Travis Longcore (who run the Urban Wildlands Group, a nonprofit conservation organization in Los Angeles), have a passion for the aesthetic qualities of night skies free from photopollution. For my part, I share their enthusiasm for a world in which humans have a much smaller ecological footprint. But the reality is that our constant drive for development, wealth creation, and all the associated ancillary insanities of consumption results in less wilderness, less wildlife, and less peace.

Rich's love of "empty" space—which, of course, from a wildlife perspective is the antithesis of empty—where species have adapted to nocturnal life strategies shines through in her preface. In their introduction, she and Longcore cite calculations that 44% of Americans live in locations where it does not become sufficiently dark for the human eye to complete the transition from cone to rod vision (*1*). The diurnal and nocturnal components of the 24-hour cycle are now blurred across large parts of the globe (almost entirely in developed countries) because of our "need" for light. The editors note that nearly 19% of Earth's terrestrial surface "experiences night sky brightness that is polluted by astronomical standards." What effect are these undark skies having on the wildlife and the ecosystem functions and services on which we depend? Providing the best examination to date of this question, the book synthesizes current thinking on a topic of considerable, if often unrecognized, importance to conservation professionals. Nearly all environmental impact assessments should include an analysis of the effects of lighting, both specific to the development of a particular site and cumulative, but very few do. Our own diurnal perspective on life blinds us, and so we forget the vast number of species that rely on darkness—to hide, to catch prey, to mate, to interact.

The book provides the scientific foundation for understanding the impacts of night lighting and then acting on research findings to reduce or, better still, avoid its damaging effects on wildlife. Although the first review of the mechanisms by which animals are attracted to lights appeared in 1958 (*2*) and its author coined the term "photopollution" in 1985 (*3*), only within the last decade has there been much research on the ecological consequences. Bearing in mind that (as noted in the book) humans have long influenced animal behavior with light (for example, the use of campfires to keep predators at bay), the dramatic increase in electrical lighting in the past 40 years is a relatively rapid change for wildlife to accommodate.

For such a new area of research, the work is fairly thorough, and the book provides many useful pointers for management. For example, road lighting may not deter vehicle collisions with mammals, and may even exacerbate the problem, because many nocturnal mammals use only the rod system for sight and bright lighting saturates their retinas. In contrast, some species of bats seem to benefit from street lighting, as they preferentially feed on insects attracted to lights, although these favored bats may in turn displace other insectivorous species that do not forage at lights through interspecific competition. Jens Rydell concludes that the replacement of mercury vapor lamps with high-pressure sodium vapor lamps (which attract fewer insects) benefits both bats and insects.

Sidney Gauthreaux and Carroll Belser's consideration of the effects of lighting on migrating birds makes particularly pertinent reading. They find that the increasing use of artificial lighting is having an adverse effect on bird populations, especially on species that typically migrate at night. Mass mortalities of birds attracted to lights were noted at lighthouses and lightships in the mid-1800s, but the relatively recent expansion of cities, the escalating height of lit buildings, and the ongoing spread of communications towers across the land are having an increasingly damaging impact on birds. Aircraft warning lights placed on such towers lead to the deaths of hundreds of thousands of nocturnal migrants each year. Most mortality occurs on nights when the moon is new or only slightly illuminated. The authors describe practical measures—such as replacing red lights, which disorientate birds, with white—with the potential for substantially reducing such losses of migrating birds.

Sea turtles are another taxon for which the effects of artificial lighting are comparatively well studied. Michael Salmon's review of research in Florida suggests the benefits of using embedded road lights (rather than poled streetlights) and replacing traditional coastal lighting, which attracts and tragically disorients turtle hatchlings. The message is clear—keep the nesting beaches dark at night.

R. N. Cohen's *Wolf Moon* (detail).

CREDIT: R. N. COHEN/EXCHANGE STREET GALLERY, PORTLAND, MAINE

Through their examples and discussions, the individual chapters provide consistently intriguing analyses that demonstrate the wide impact of light pollution. So much of the book is of direct relevance to the environmental advice we try to give in the United Kingdom that I expect it will be helpful around the globe. *Ecological Consequences of Artificial Night Lighting* is an excellent reference that will undoubtedly raise awareness of the need to conserve energy, do proper impact assessments, and turn the lights down.

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VERTEBRATE PALEONTOLOGY

True Giants on Earth

David Norman

Despite the fame and Hollywood-inspired notoriety of the predatory theropod *Tyrannosaurus*, it is really the herbivorous sauropods, named by Othniel Charles Marsh in 1878, that are more truly the epitome of the dinosaur: gargantuan creatures with improbably long tails and necks, the latter capped by an absurdly small head. Paleontologists as eminent as Alfred Sherwood Romer were driven to despair by the group. In 1968, he predicted, "It will be a long time, if ever, before we obtain a valid, comprehensive picture of sauropod classification and phylogeny" (*1*).

During the time of Romerian despair, one man, Jack McIntosh (by profession a physicist at the Wesleyan University in Connecticut), developed an all-consuming passion for these animals. He spent years documenting their remains and the history of their discovery, radiating enthusiasm for them at scientific meetings, and energetically encouraging any young paleontologists that he met to work on "his" charges. Although never professing any great knowledge of his subjects, and embarrassingly deferential to supposed paleontological experts, he was instrumental in putting the correct head on *Apatosaurus* (formerly known as *Brontosaurus*) and correcting many errors in our understanding of the group as a whole. It is, however, as an unsung hero and father to a younger generation of sauropodologists that he is now being recognized in *The Sauropods*. Editors Kristina Curry Rogers (at the Science Museum of Minnesota) and Jeffrey Wilson (at the University of Michigan) are an integral part of the new generation of paleontologists that have heeded McIntosh's pleas and urgings.

What is achieved in the volume is a formal end to Romer's despairing commentary. Here is a detailed consideration of the formal classification and phylogenetic analyses of Sauropoda and much more besides. Since the 1970s, the entire field of dinosaur paleontology has been invigorated by a paleobiological approach (a reinvention of the *paleobiologie* of the 1920s). Systematic studies using cladistic methods have generated testable phylogenetic hypotheses, which permeate all the chapters in the first half of the book. These set a systematic framework that is used to dissect the evolutionary relationships within the clade Sauropoda that have proved so problematic over the years. The studies also provide starting points for more detailed investigations of the mode and tempo of the group's evolution, especially in the contexts of Mesozoic geological history and events that affected the faunas encompassing these dinosaurs.

The chapters in the second half of the book focus on sauropod biology. These include informative studies of the mechanical design of the animals' preposterous bodies. The flexibility of the backbone, for example, extended the sauropods' browsing range compared with that of modern herbivores. The evidence for pervasive skeletal pneumaticity (a birdlike air-sac system associated with the lungs) of these dinosaurs indicates that they were probably not as heavy as they outwardly appeared. Two contributors focus on the limbs: the limitations imposed on the range and variety of locomotor systems and the importance of sauropod tracks in understanding how, when, and where they moved. There are also interesting observations on sauropod ontogeny using evidence from bone histology and embryos in eggs.

The editors and contributors clearly intend the volume to add materially to the scientific debate and generally to increase the profile of sauropods, and it should do both. It also serves to supplement the sauropod section of the recent edition of the massive compilation *The Dinosauria* (*2*), which updates McIntosh's contribution to the original edition (*3*). But as a dinosaur paleobiologist, books of this particular type leave me feeling faintly uneasy. This is clearly a festschrift volume that might, more appropriately, have appeared in a scientific journal. Each chapter has the language and format of a normal scientific paper (minus the abstract), and many seem to have undergone some form of critical evaluation. However, not all of the material is new and original; much represents the reworking of earlier papers.

There is also a more pernicious matter, one relating to first impressions. The dust jacket, with its painting portraying nestling titanosaurids and their (apparently doting) parents, might suggest that this is actually a children's book. The problem is probably my own, but I share it because I believe that it is an effect that undermines the science associated with dinosaur paleobiology. Although I am sure that the publisher's marketing department sees profitability in aiming the book toward the dinosaur-enchanted public, image and marketing should not outweigh content. If judged by its cover, the book will not be placed alongside other academic titles (where it clearly belongs) but in the family-children's section.

The book concludes with edited highlights of conversations the editors held with the dedicatee in 2004, which allow an all-too-brief glimpse of a deeply modest man whose life span has allowed him to rub shoulders with some of the greats in paleontology. Overall *The Sauropods* is a useful addition to dinosaur paleobiology. The editors have drawn together a variety of approaches that show how a rigorous scientific approach to these genuinely daunting animals provides insights into the biology and natural history of organisms that lived more than 70 million years ago. McIntosh will no doubt be simultaneously thoroughly embarrassed by such recognition and yet tickled pink to see his sauropods receiving such a range of scholarly attention.

The Sauropods
Evolution and Paleobiology

Kristina A. Curry Rogers and Jeffrey A. Wilson, Eds.

University of California Press, Berkeley, CA, 2005. 359 pp. $65, £41.95. ISBN 0-520-24623-3.

Bending limits. Kent Stevens and J. Michael Parrish used a parametric skeletal modeling approach, DinoMorph, to estimate the limits of neck flexibility in *Apatosaurus louisae*.

CREDIT: FIG. 6.10 IN THE SAUROPODS

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10.1126/science.1126250

PRIORITIES AND FUNDING

"Knowledge Innovation" and the Chinese Academy of Sciences

CAS is working to set the course for scientific and technological development over the next 15 years.

Richard P. Suttmeier,[1]* Cong Cao[1,2], Denis Fred Simon[2]

At China's 2006 National Science and Technology Conference, President Hu Jintao pledged to make 21st-century China "an innovation-oriented society." To that end, the conference unveiled a 15-year Medium to Long-Term Science and Technology Development Plan (MLP) (2006–2020) setting national research priorities and providing substantial resources for meeting them. Gross expenditures on R&D (GERD) are expected to rise to 2.5% of the gross domestic product (GDP) at the end of the plan period from its 2005 level of 1.30% (*1*). The plan emphasizes "indigenous innovation," and "leapfrogging" in research. Science and technology are expected to support and lead future economic growth.

Behind this new plan is a complex story of 20 years of policy development and institutional reform. This is illustrated in the experiences of the Chinese Academy of Sciences (CAS) and its efforts to reinvent itself through the "Knowledge Innovation Program" (KIP) (*2*). A review of CAS can help explain forces driving the scientific infrastructure and challenges in the new long-term plan.

PRIORITY MISSION AREAS FOR CAS

Information technology
Optical electronics, space science, and technology
Advanced energy technologies
Materials science, nanotechnology, advanced manufacturing
Population, health, medical innovation
Advanced industrial biotechnology
Sustainable agriculture
Ecology, environmental protection
Natural resources, ocean technologies
Comprehensive research relying on megascience facilities

Objectives and Achievements

In 1998, when KIP was initiated, CAS supported 120 institutes, many of which had overlapping missions and outdated research agendas. Most institutes were overstaffed with nonresearch personnel and had more than their share of scientists who had passed their peak productivity and lagged behind international research frontiers. Research programs were often derivative of foreign science, physical facilities were typically run down, and the quality of equipment was very uneven. To attack these problems, one of KIP's main goals is creation of 30 internationally recognized research institutes by 2010, with five recognized as world leaders.

Between 1998 and 2005 the number of institutes was scaled back to 89 as a result of converting some applied research institutes into commercial entities and the reorganization of others to reduce duplication and rationalize missions. At individual institutes, traditional disciplinary orientations and missions have been redefined and more focused.

Revitalization of the human resource base in CAS has been approached by recruitment of talented group and laboratory leaders from "brain drain" scientists working abroad and from young researchers in China. The "100 Talents" Program, for instance, offers high salaries, responsible positions, and generous start-up research support to promising scientists under 45 years old (*3*). Between 1998 and 2004, 899 researchers were recruited using this mechanism, 778 of whom were working overseas (392 of these had doctorates from foreign universities). The academy also expanded its graduate training, with total enrollment as of the end of 2004 reaching some 33,000 at its institutes, its graduate school, and its University of Science and Technology campus. A CAS university center in Beijing is now under construction.

The average age of institute directors and deputy directors in 1991 has dropped from 56 in 1991 to 47 in 2003. Between 1998 and 2003, CAS made 14,409 new appointments, 67.8% of whom were senior scientists under the age of 45 (*4*). New appointments no longer carry promises of lifetime tenure but are subject to evaluation early in the investigator's career. Salary structures have also changed and now include provisions for merit increases.

In the past 7 years, KIP has provided project support in fundamental research, technologies with strategic significance, and science and technology for managing resources and the environment. The pattern of KIP funding, with 70% going directly to institutes and 30% controlled by CAS headquarters, has given institutes considerably more discretion in research management. Additions of KIP funds to institute budgets have made CAS institutes more competitive vis-à-vis universities and other government research institutes for grants and contracts. CAS research outputs (publications in *Science Citation Index*–catalogued journals, patents granted, and copyrights registered) have increased by more than an order of magnitude.

KIP implementation has been accompanied by the introduction of a demanding evaluation system. It involves administrative reviews to assess the consistency of institute activity with CAS policy and KIP objectives, as well as peer review of professional work by leading Chinese and foreign scientists. There has also been a major investment in upgrading facilities and equipment. CAS manages most of China's megascience facilities, and substantial investments are shown by the construction of the Large Sky Area Multi-Object Fiber Spectroscopic Telescope (LAMOST) astronomical telescope; the reconstruction of the Beijing Electron Positron Collider (BEPC); the Lanzhou Heavy Ion Accelerator; the Synchrotron Radiation Facility and the Controlled Nuclear Fusion Device, both in Hefei; and construction of the Shanghai Synchrotron Radiation Facility. CAS also continues to play a key role in China's defense establishment, participating in everything from the space program to supercomputer development.

The Challenges Ahead

During the 2005–2010 period, CAS seeks to respond to emerging national policy priorities, including those identified in the national 11th Five-Year Plan and the new MLP, and secure its place as the "backbone" of the national system of innovation. To these ends, it is establishing a "1+10" strategy, in which activities of its research institutes will be linked to 10 mission objectives (see table, above). A commitment to interdisciplinary basic research in frontier areas will support the effort. This strategy requires administrative reorganization within CAS that will have implications for relations between the institutes and CAS leadership.

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Human resources. Although CAS has sought to recruit the very best scientific talent, its success has been mixed. Some Chinese scientists working abroad have joint appointments in CAS, but it has been difficult to attract back on a permanent basis those who are most active at the frontiers of international science. Indeed, it is the latter group of scientists that has become more vocal in their criticisms of the Chinese research environment (*5*–*7*). In addition, CAS is still losing many of its top students to study and research opportunities abroad and to alternative employment opportunities in China, including work in universities and in the growing number of R&D facilities operated by multinational corporations. Within the CAS graduate school system, the steady expansion of enrollment brings to the fore the question of maintaining quality control (*8*).

High-quality Chinese researchers expect a degree of stability and autonomy in the research environment and worry that the new initiatives could be a threat. The evaluation system, especially for new group leaders, generates enormous pressures for productivity. In some cases, this pressure has caused promising scientists to leave CAS for employment elsewhere.

Different types of evaluation standards and processes will need to be developed. CAS aspirations to achieve world-class research status will put a premium on scientific distinction. However, with increased funding, CAS faces new problems of political accountability and government expectations that national needs are being served cost-effectively. This may require evaluation to focus more on consistency with national policy and on the extent to which social needs are met. Imposition of excessive top-down requirements on the research community could discourage creativity and bottom-up innovation. A failure to fine-tune the evaluation system to meet multiple objectives may lead to dissatisfaction from all quarters.

Institutional mission and focus. Few institutions in the world incorporate in one organizational framework so many different activities and goals: basic research; cutting-edge R&D; "public goods" research programs in agriculture, health, energy, and the environment; sponsorship of graduate training; and operation of more than 400 hundred companies, in cooperation with local governments. Finally, its elite "academicians" (*yuanshi*) have important science advisory functions, although publicized abuses have made the system increasingly controversial (*9*). The multiple functions that CAS assumes can threaten maintenance of clear organizational focus. A case might be made for greater specialization and functional differentiation within the organization.

CAS and the National System of Innovation (NIS). As China has moved from a planned to a market economy, there is a growing realization among policy-makers that Chinese industry must become far more innovative. As a result, government policy has recently favored the expanded development of research in business enterprises, with more than 60% of the nation's R&D reportedly now supported by industry (*10*). The importance of building an "enterprise-centered NIS" was reaffirmed in the MLP, and proindustry policy measures will be introduced to make it a reality over the next 15 years.

CAS is faced with the challenge of reconciling its view of itself as the backbone of the nation's innovation system with this "enterprise-centered" model. On the basis of current trends, it is unlikely that many Chinese companies will develop R&D capabilities in support of novel, science-based technologies in the near term. China's more entrepreneurial high-technology companies often lack resources to support their own R&D. Larger state-owned enterprises often find that short-term business objectives are better met by the less risky course of procuring advanced technology from abroad. CAS represents a reservoir of assets for research and innovation. How it makes these assets available to the companies that will actually be marketing products and services is one of the major challenges in making the "innovation-oriented society" a reality.

Although, historically, CAS has been weak in its service to industry, the commercial pressures it has faced over the past 20 years have produced a variety of transfer mechanisms. These include contract research, the licensing of proprietary technologies, the spinning off of new companies from CAS institutes, and the establishment of CAS facilities to serve industry in special high-technology zones established by local governments (*11*). However, problems still remain. There are often mismatches between the relatively advanced technologies being developed by CAS and the willingness and ability of Chinese companies to adopt them. Some CAS researchers are concerned that industrial outreach takes the academy too far downstream (and away from its core strengths) in the innovation process.

Public goods (e.g., public health, agriculture, defense, weather forecasting, and environmental protection) require technology transfer platforms that involve cooperation with other state bureaucratic systems (that have their own research establishments and actually compete for funding with CAS). Relations with local governments may be useful, but they are no substitute for deployment of substantial managerial resources and interagency coordination. Too much involvement with local governments is seen by some in CAS as diverting attention away from its broader, national mission.

Chinese universities had a limited research role in the past, but the value of associating research with graduate education, characteristic of the Western model, has taken root. The role of CAS in relation to universities has become a more pressing issue, especially with regard to sharing of facilities and staff, training and subsequent employment of graduate students, and leadership roles in high priority areas of research.

CAS faces a series of questions as it moves to the next phase of KIP. Do its strategies (including funding and evaluation systems) encourage development of a culture of creativity where risk-taking, initiative, and new ideas are supported and rewarded? Can CAS develop R&D managers with the skills and training for managing interdisciplinary teams in an increasingly international environment? How should CAS set priorities related to its stakeholders, and develop an organizational structure that fits diverse needs? Should it define its mission principally in terms of the supply of public or private goods, and how does it define "success"? How can its educational mission meet its own needs and complement the activities of Chinese universities? In its commitment to serve national needs, can it also be a credible international partner? In its efforts to reinvent itself, CAS still faces formidable problems of internal management and building new relations with the broader national innovation system. Despite these, the trajectory set by KIP helps ensure a central role for CAS in China's emergence as a major player in international research and innovation.

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SOCIAL SCIENCE

Cooperation, Punishment, and the Evolution of Human Institutions

Given the choice, people prefer institutional arrangements in which those who overconsume common-property resources are punished compared to those in which they go free.

Joseph Henrich

Explaining the scale, diversity, and historical dynamics of human cooperation is increasingly bringing together diverse empirical and theoretical approaches. For decades, this challenge has energized evolutionary and economic researchers to ask: Under what conditions will decision-makers sacrifice their own narrow self-interest to help others? Although classic evolutionary models based on relatedness and reciprocity have explained substantial swaths of the cooperation observed in many species, including our own, theoretical work in the 1980s demonstrated that the puzzle of cooperation in large groups, or in situations without much repeated interaction, remained unsolved and would likely require alterative theoretical formulations (*1, 2*).

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Such cooperative dilemmas, or "public goods" problems, involve situations in which individuals incur a cost to create a benefit for the group. In our society, think of recycling, buying a hybrid car, valor in combat, voting, and donating blood. The dilemma arises from free-riders who enjoy the group benefits created by the contributions of others without paying the costs. Even if nearly everyone is initially cooperative and contributes, free-riders can profit and proliferate, leading to the eventual collapse of cooperation. So, understanding how public goods problems can be solved has provoked great interest, both because human societies have somehow managed to solve many such problems to varying degrees, and because some of the world's most pressing issues, such as global climate change, are essentially public goods dilemmas. On page 108 of this issue, Gürerk *et al*. (*3*) take an important step in understanding how self-sustaining cooperative institutions may have emerged over the course of human history.

Free-riders not wanted. Those who do not contribute but benefit from the efforts of others can cause the collapse of cooperation. Groups that sanction such free-riders stabilize cooperative behavior and outcompete groups that do not.

Recent models have demonstrated how evolutionary processes (genetic or cultural) can maintain cooperation in large groups or without repeated interaction. Costly signaling models have shown how cooperation by "high-quality individuals" (those who are potentially desirable as allies or mates) can be sustained if such individuals can accurately signal their quality by making substantial cooperative contributions to public goods (*4*). For example, great hunters might supply all the meat for a public feast, or millionaires might donate a recreational center to their community. Similarly, reputation-based models have shown how cooperation can be sustained if individuals' reputations for not contributing to public goods reduce their payoffs (or fitness) by altering how others treat them in certain dyadic social interactions (*5*). Finally, models that allow individuals to both contribute to the public good and to sanction noncontributors have revealed stable cooperative solutions, especially when the strategies for cooperation and punishment are influenced by social learning (*6*). Thus, a number of possible stable solutions to the puzzle of cooperation in large groups, or cooperation without repeated interaction, have now emerged.

It turns out, however, that finding a stable solution is only the first step in confronting the dilemma of cooperation. Each of the above approaches can actually stabilize any behavior or practice, independent of whether it delivers any benefit to anyone. This includes behaviors that reduce the payoff or fitness of the group. For example, instead of public goods contributions, costly signaling could maintain behaviors involving dangerous physical feats (like scaling icy mountain peaks), aggressive displays (like beating up your neighbor), or extravagantly wasteful feasts. Similarly, the same reputational and sanctioning mechanisms that can stabilize cooperation can also sustain maladaptive practices such as consuming the brains of dead relatives, flattening the foreheads of infants, or binding the feet of young girls. Thus, there are actually a multitude of stable equilibria, only some of which are cooperative. What determines which equilibria emerge and/or spread?

Three broad theoretical approaches confront the problem of equilibrium selection. The first, and perhaps the most intuitive, is that rational, forward-looking individuals recognize the long-term payoffs available at stable cooperative equilibria, assume others are similarly sensible, and choose the cooperative state (*7*). The second approach is based on the stochasticity inherent in any interaction. Different stable equilibria are more or less susceptible to this stochasticity, meaning that in the long-run, some equilibria will be substantially more common than others (*8*). The third mechanism, cultural group selection, gives priority to the competition among social groups who have arrived at different culturally evolved equilibria. This intergroup competition favors the spread of individuals and practices from groups stabilized at more cooperative equilibria. In humans, competition between groups can take the form of warfare, demographic production (some social groups reproduce faster than others), or more subtle forms in which individuals learn decisions and strategies by

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preferentially observing more successful individuals, many of whom are more successful because they live in groups at stable cooperative equilibria (*9*). This can lead to a flow of decisions, strategies, and even preferences from more cooperative groups to less cooperative ones (*6*), or to a migration of individuals among groups (*10*) that favors the spread of the more cooperative equilibria.

Gürerk *et al*. address the issue of equilibrium selection with an elegant addition to the existing experimental work on public goods. In their experiment, individuals (the "players") choose between two different "institutions." In one institution, players can contribute money to a group project. The sum of all contributions to the project is augmented by a fixed percentage and then is divided equally among all players, regardless of their contributions. Previous experiments established that when this interaction is repeated, mean contributions to the public good drop to near zero (a noncooperative equilibrium). The other "sanctioning" institution is very similar, except that after players have contributed, they can pay to punish (reduce the payoff of) other players. When this interaction is played repeatedly (*11*) a substantial fraction of players punish low contributors, causing mean contributions to rise and stabilize near full cooperation (a cooperative equilibrium). Both institutions were run concurrently for 30 interactions and players could, initially and after each subsequent interaction (after seeing others' payoffs), choose their institution for the next interaction.

The principal findings of Gürerk *et al*. can be summarized simply. Initially, most players picked the institution without sanctioning possibilities. But, as usual, free-riders in the nonsanctioning institution started driving mean contributions downward, so cooperators, who hate being exploited by free-riders, started reducing their contributions. Meanwhile, in the sanctioning institution, punishers started driving contributions up by inflicting costs on noncontributors, despite the personal cost of punishing. After a few interactions, players from the nonsanctioning institution—presumably seeing the higher payoffs of those choosing the sanctioning institution—increasingly switched institutions. Notably, despite the incoming flow of migrants from the nonsanctioning institution, the mean contributions in the sanctioning institution consistently increased or held stable near full cooperation. In fact, most incoming migrants, consistent with local norms in their new setting, increased their contributions during their first interaction in the sanctioning institution, and a majority administered some punishment.

What does this tell us about equilibrium selection? First, the players' degree of rationality did not permit them to foresee the final outcome and select the higher payoff institution on the first interaction. Second, despite the stochasticity of human decisions, neither institution drifted to another equilibrium. What did happen is that once players from the lower payoff institution observed the higher payoffs of the other institution, they wanted to adopt either the practices of the higher payoff institution, or the decisions and strategies of those other players. Consistent with ethnographic and historical case studies (*12*, *13*), the present work provides an important experimental demonstration of cultural group selection in action, as the two alternative equilibria compete for shares of the total population.

The course charted by Gürerk *et al*. should spur more empirical work on how processes of equilibrium selection influence the evolution of institutional forms. Many questions remain to be tackled: for example, what happens if switching institutions is costly, or if information about the payoffs in the other institution is poor? Or, what happens if individuals cannot migrate between institutions, but instead can vote on adopting alternative institutional modifications? Such work can both help us understand how humans became such a cooperative species, and teach us how to build durable cooperative institutions that solve public goods problems and are readily spread.

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EVOLUTION

Reducible Complexity

Christoph Adami

How does biological complexity arise? The molecular evolution of two hormone receptors was traced from a common ancestral receptor. Through a series of mutations, receptors with distinct hormone binding properties evolved, one before the appearance of its cognate ligand.

If an elaborate lock fits an equally elaborate key, we immediately sense the purpose of design: The key was crafted with the idea of the lock in mind. We would not entertain the possibility that the match is accidental. When we come upon such lock-and-key pairs in nature, it is natural to ask how these pairs could have evolved via Darwinian evolution. At first glance, it seems that the key can only evolve to fit the lock if the lock is already present, and the lock cannot evolve except in the presence of the key (because without the key, it does not open). On page 97 of this issue, Bridgham *et al.* (*1*) take a closer look at this puzzle and discover a different answer in the molecular evolution of hormone-receptor interactions.

Charles Darwin was fully aware of the problems that such lock-and-key systems—should they exist in biology—would present to his theory because the theory relies upon step-by-step changes to a trait. Building a lock-and-key system appears to require at least two changes to happen simultaneously. He famously remarked that "if it could be demonstrated that any complex organ existed which could not possibly have been formed by numerous successive slight modifications, my theory would absolutely break down" (*2*). This concern has been seized upon by proponents of an "intelligent design" alternative to Darwinian evolution that proposes that complex systems—like those that display lock-and-key complexity—cannot evolve. The premise for the argument is that systems of a lock-and-key nature cannot evolve and are thus "irreducibly complex" (*3*), implying that only the lock-and-key combination, but not its parts, is complex. The argument continues that because such systems do exist in nature, and cannot have evolved, they must have been "designed."

Darwin already saw how such thorny issues could be resolved. He further explains in *The Origin of Species* that "if we look to an organ common to all the members of a large class…in order to discover the early transi-

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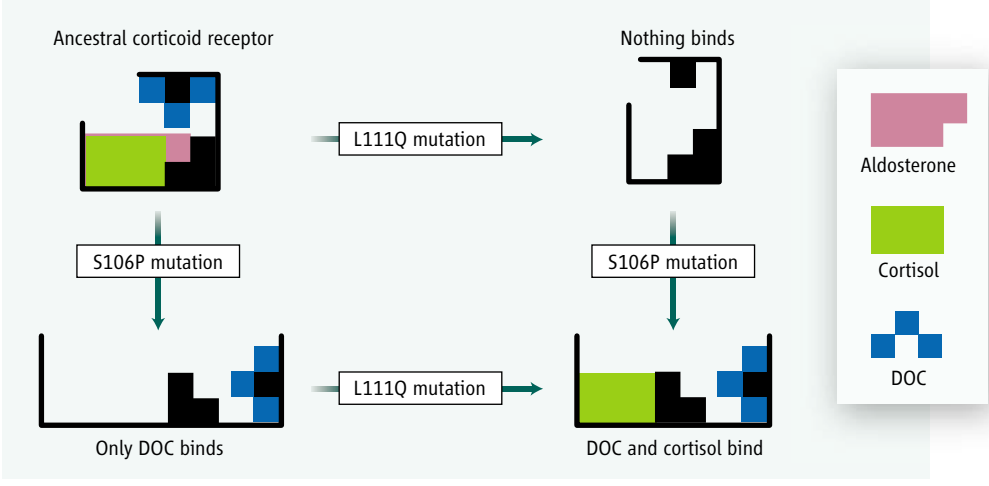
tional grades through which the organ has passed, we should have to look to very ancient ancestral forms, long since become extinct." In other words, Darwin suspected that viewing only the extant complex forms will obscure the path of evolution, and present an incomplete picture. But while the fossil record has yielded many intermediate forms that suggest a continuous evolution of traits, it is too often incomplete, and does not allow us to retrace the molecular history of a gene. Reconstructing the complete evolutionary history of a complex genetically encoded function (albeit a "computational" one) was achieved recently (*4*), and it experimentally vindicated Darwin's idea that the target of natural selection constantly changes, so that the complex feature of today may share very little with the original function. But while such computational investigations can be very satisfying, they might not convince everybody. It is therefore gratifying that it is now possible to reconstruct the ancestral genes of an existing species so that, as Darwin urged us to do, we can "look exclusively to its lineal ancestors" to understand a gene's evolution.

Bridgham *et al*. address one of the central concepts of the intelligent design argument. They did not study just any gene, but precisely a system that looks irreducibly complex: a hormone-receptor pair that we can think of as a biological lock and key. In vertebrates, the regulation of many cellular processes is controlled by steroid-receptor interactions that are highly specific. For example, cortisol activates the glucocorticoid receptor to regulate metabolism, inflammation, and immunity. In contrast, the mineralocorticoid receptor is activated by aldosterone, and controls electrolyte homeostasis, among other effects. This specificity is important, because the activation of the glucocorticoid receptor by aldosterone, for example, would be highly detrimental.

Phylogeny tells us that an ancestral corticoid receptor gave rise to the glucocorticoid receptor and the mineralocorticoid receptor in a gene-duplication event more than 450 million years ago. However, aldosterone evolved much later. Without aldosterone present, how could the mineralocorticoid receptor evolve to be activated by it? Doesn't the pair's specificity require the evolution of two traits at the same time, an event that appears highly unlikely?

Bridgham *et al*. took Darwin's advice and followed the line of descent to the ancestral corticoid receptor. Modern phylogenetic methods make it possible to reconstruct such inferred sequences and study the properties of these molecules in the laboratory. What the authors find is a surprise: Not only is the ancestral corticoid receptor sensitive to cortisol as expected, it is also activated by 11-deoxycorticosterone (DOC) and aldosterone. Because aldosterone was not present at the time, this sensitivity must be a by-product of sensitivity to another steroid, a promiscuity that can be exploited by evolution (*5*).

The next task was to determine how the mineralocorticoid receptor kept the aldosterone specificity, whereas the glucocorticoid receptor lost it. This is a tale of two mutations. More phylogenetic analysis revealed that precisely two amino acid substitutions resulted in the glucocorticoid receptor phenotype—aldosterone insensitivity and cortisol (and DOC) sensitivity. Could these two mutations have occurred one after the other? Bridgham *et al*. tested the effect of each of these mutations—replacement of leucine-111 with glutamine (L111Q) and replacement of serine-106 with proline (S106P)—alone on the reconstructed ancestral corticoid receptor and in the presence of the other mutation (see the figure). Of the two mutations, L111Q was the more damaging: Applying this mutation to the ancestral receptor destroyed its sensitivity to all three hormones. On the other hand, the S106P change reduced receptor activation by aldosterone and cortisol but did not change the sensitivity to DOC. In the presence of S106P, the effect of L111Q was quite different: It removed any sensitivity to aldosterone, and restored cortisol sensitivity. In other words, it produced the glucocorticoid receptor phenotype. The two mutations thus turned out to be strongly epistatic: Both reduce the fitness of the system (L111Q very strongly so), but together their effect is neutral or better.



Molecular evolution of a biological lock and key. A two-dimensional schematic picture of an ancestral hormone receptor that binds aldosterone, cortisol, and DOC. The L111Q mutation in the receptor is drastic because it eliminates receptor activation by any of the three molecules, modeled by an obstruction of the binding pocket. The mutation S106P, on the other hand, does not affect the binding of DOC, but both aldosterone and cortisol can bind only very loosely. However, the presence of both mutations allows cortisol to bind strongly again, whereas aldosterone no longer fits.

Can we determine the order in which these mutations appeared and can we understand how such epistatic effects arise? Structural changes very easily can lead to the type of epistatic interactions between mutations now documented in hormone receptor evolution, because such changes can condition the mutational effect. Thus, single mutations that confer different structural changes that depend on one another can conspire to give the impression of irreducible complexity. Although the mutation L111Q creates a possibly lethal phenotype when it occurs alone in the ancestral corticoid receptor, it confers the glucocorticoid receptor phenotype if it is preceded by the S106P mutation, which itself is nonlethal. Such interacting pairs of mutations are common and important in evolution.

Bridgham *et al*. conclude that the insensitivity of the glucocorticoid receptor to aldosterone most likely evolved by the S106P mutation followed by the L111Q mutation because the intermediate phenotype is still viable. Although this is the most parsimonious conclusion, the other sequence of mutation events cannot be ruled out. Indeed, the experiments following the line of descent of digital organisms in Lenski *et al*. (*3*) found, surprisingly, that occasional highly deleterious mutations were rescued by a partner mutation that conferred a beneficial trait. Thus, the highly deleterious partner of the pair can indeed come first, as long as the second mutation does not occur too late. In any case, the evidence is clear that such "multiresidue features" (*6*) can and do evolve. Understanding how they evolve requires taking into account

complex epistatic interactions that allow intermediate nonlethal states that might not appear obvious at first glance.

The Bridgham *et al.* and Lenski *et al.* (*4*) studies are of particular scientific interest, given the political attention given to intelligent design lately. Although these authors have not directly addressed this controversy in the discussion of their work—because the work itself is intrinsically interesting to biologists—such studies solidly refute all parts of the intelligent design argument. Those "alternate" ideas, unlike the hypotheses investigated in these papers, remain thoroughly untested. Consequently, whatever debate remains must be characterized as purely political.

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PHYSICS

New Additions to the Schrödinger Cat Family

Schrödinger cat states entail superpositions of seemingly opposite quantum states, metaphorically like a cat being both dead and alive. Femotsecond laser pulses can now induce photons into small and unbound Schrödinger kitten states.

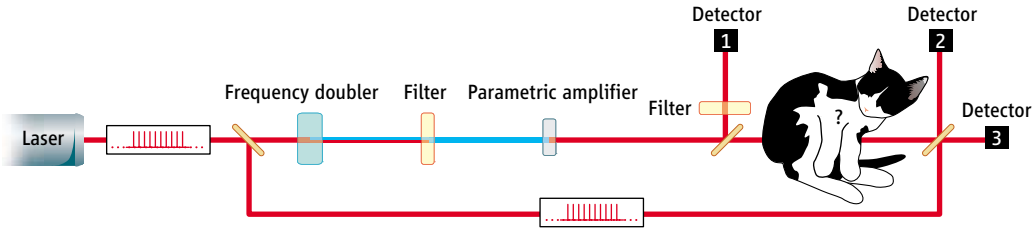
Nicolas Gisin

Can a cat be simultaneously dead and alive? Before the era of quantum physics, the answer would have been obvious to any reasonable person. But quantum physics is well known for being counterintuitive, as beautifully exemplified by Schrödinger's cat. In this famous example, a cat is hidden in a box and we do not know whether it is dead or alive until we make a measurement (by opening the box). According to quantum physics, the cat must exist in a quantum superposition of the "dead" and "alive" states—the cat is "dead-and-alive." Currently, experiments involving Schrödinger cats are still Gedanken experiments; however, technology is making huge progress. Recently, some states of the electromagnetic field mimicking small Schrödinger cats have been realized in optical cavities (*1*). As reported on page 83 of this issue, Ourjoumtsev *et al.* (*2*) have added to this strange family of quantum cats by creating flying Schrödinger kittens.

Historically, Schrödinger used his cat example to stress the oddness of quantum physics. In Schrödinger's opinion, superpositions of macroscopically distinguishable states could not exist. His example was thus presented as an argument against the completeness of quantum mechanics. Since Schrödinger's time in the 1930s, this remained a philosophical issue. But, in the past decade or so, physicists have made many of advances. On the theoretical side, it was understood that the main difficulty in producing Schrödinger cat–like states is decoherence, a phenomenon that quickly destroys the superposition of large objects if they are not perfectly isolated: The larger the object, the better it must be isolated to behave quantum mechanically. Decoherence doesn't answer all of the questions about cat states, and in particular, it doesn't help us understand the uniqueness of quantum measurement results. But it does answer qualitatively and quantitatively why Schrödinger cats are so fragile. An object twice as large must be exponentially better isolated. Understanding decoherence helps us to find ways around this problem. On the technology side, the new science of quantum information has given a huge impetus to new developments toward mastering individual quantum phenomena. Indeed, such mastery will open revolutionary new ways for information processing (*3*).

No physicist is really thinking of superposing actual cats, not even kittens. Any macroscopic system, or a mesoscopic system for that matter, would suffice to fill the entire physics community and beyond with wonder. In particular, it would suffice to demonstrate the superposition of a light pulse in a superposition of being "here" and "there." This should not be confused with a light pulse as it passes through a beam splitter. In this case, half of the pulse is transmitted and half is reflected, which is nothing strange (that is, as long as one doesn't think of the pulse as being made out of many photons, each photon in a superposition state of transmitted and reflected, an example of basic quantum strangeness). In contrast to a pulse passing through a beam splitter, a Schrödinger cat light pulse is a pulse that is entirely transmitted (with zero intensity reflected), superposed (that is, coexisting) with a pulse that is entirely reflected (with zero intensity transmitted).

In their effort to study cat states, Ourjoumtsev and colleagues from the Optics Institute in Orsay, near Paris, present a remarkable experiment (see the figure). They produced a (very small) Schrödinger kitten in the form of a tiny light pulse. To achieve this, they first pumped a nonlinear crystal to produce a light pulse of 180-fs duration, and this pulse has some special properties. Namely, the pulse contains photons that are quantum entangled in the way described by Einstein, Podolsky, and Rosen (EPR) more than 70 years ago. Next, they cleverly removed precisely one photon from the pulse.



Flying kittens. Simplified version of the experiment. A femtosecond laser creates a train of red pulses that are frequency doubled to create blue pulses. After the red is filtered out, the blue pulses are fed into a nonlinear crystal that "squeezes" the light into an EPR state. A single-photon detector (1) signals when one photon has been removed from the pulse by the beam splitter, and thus marks the creation of a quantum kitten. More detectors (2 and 3) are used to study the properties of the kitten pulse.

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This results, to a very good approximation, in a small Schrödinger kitten state of the remaining light pulse. Finally, they used a high-quality homodyne detector to measure the state of the pulse (more precisely, its Wigner function, which is a special kind of probablity distribution in phase space). The mean photon number in the pulse is slightly less than one. This seems disappointingly small. However, the light pulse is in a coherent state (more precisely, in a superposition of two coherent states), and coherent states are themselves superpositions of states with arbitrarily high photon numbers. In order to compare their results with simulations, the authors had to expand the coherent states up to photon-number states with five photons.

Five photons may still seem like a very dim pulse, but it is actually bright enough to be seen with the naked eye (when at the optimal wavelength). Furthermore, the theorists know how to breed Schrödinger cats from kittens using linear optics. Hence, in principle, the race toward larger and larger Schrödinger cat states is on, although the technical path is full of pitfalls. The question of whether there is a fundamental size limit to Schrödinger cat states remains open.

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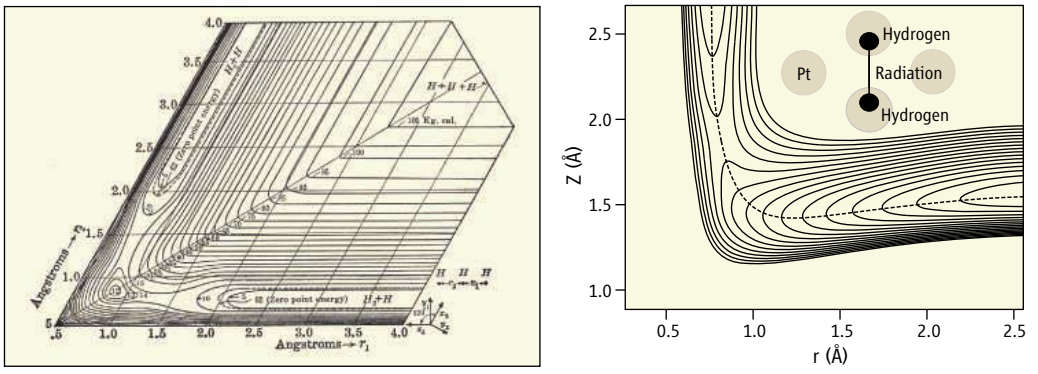
CHEMISTRY

Chemistry in a Computer: Advancing the in Silico Dream

Alec M. Wodtke

Theoretical studies of chemical reactions often make the simplifying assumption that the motion of atoms is not coupled to the motion of their electrons. While this assumption is questioned for reactions on metal surfaces, it is useful for describing hydrogen reactions on platinum.

Imagine that laboratory chemists might one day sit down at a computer, punch in data characterizing a new reaction of potential industrial importance, and receive computer output describing the likely reaction rates, temperature and pressure conditions required, and possible catalysts that would speed up the reaction rate to useful speeds. Although still only a dream, such a tool is beginning to take shape, at least in a rudimentary form. Indeed, as Nieto *et al*. report on page 86 of this issue (*1*), we are slowly but surely reaching the long-sought goal of using first-principles theory, based firmly in quantum mechanics, to predict the properties of chemical reactions at metal surfaces. Such reactions underlie all of heterogeneous catalysis.



Reaction models. (**Left**) The first potential energy surface for a chemical reaction was devised in 1935 to explain the simplest $H + H_2 \rightarrow H_2 + H$ reaction. (**Right**) Today, theoretical chemists are able to accurately explain reactions occurring at the surface of a bulk metal, providing a quantitatively accurate look at the inner workings of reactions important to heterogeneous catalysis.

The so-called standard model of chemical reactivity was first described by Michael Polanyi and Henry Eyring in 1935, when they realized that the Born-Oppenheimer approximation could be used to dramatically simplify the solution to the many-body Schrödinger equation, the most fundamental law of quantum mechanics for chemical reactions (*2*). This brilliant insight, overlooked by the Nobel committee in perhaps the greatest oversight in the history of the chemistry prize, led Eyring and Polyani to the computational machinery that allows construction of a potential energy surface describing all of the forces between the atoms taking place in any chemical reaction (see the figure). Knowledge of the interatomic forces allows accurate computer simulations of chemical reactions, in principle providing every knowable characteristic of that reaction under any conceivable set of reaction conditions. Since that time, our methods for solving Schrödinger equations—especially the advent of density functional theory (*3*)—have advanced by leaps and bounds. Development of ever more powerful computers has accelerated the rise in importance of theoretical chemistry. Some remarkable successes include the quantitative agreement between experiment and theory for the prototypical $H + HD \rightarrow H_2 + D$ reaction (*4*) and the advent of the successful use of theoretical advice for new industrial heterogeneous catalyst development (*5*).

Understanding reactivity at solid surfaces, especially surfaces that are models for heterogeneous catalysis, is an extremely exciting forefront area of modern research. As heterogeneous catalysis is involved in about one-third of the modern economy in one way or another, how we apply modern theory to this problem has become a question of profound importance. At the heart of the standard model of reactivity is the Born-Oppenheimer approximation, which requires that electrons not be excited to higher quantum states by the motion of the atoms as they react. The physical picture that justifies this assumption is the large difference in time scales for electron motion versus atomic nuclear motion. Essentially, one assumes that the electrons are able to adjust instantaneously to the new position of the nuclei without being excited by that motion. Although this assumption has proven highly accurate for many reactions, it is inherently questionable for reactions taking place at the surface of solid metals. The Heisenberg uncertainty principle tells us that confining electrons in space drives up their translational energy. The high speeds they obtain on small isolated molecules a few

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angstroms in size ensures that they move much more rapidly than the atomic nuclei. For metallic (conduction) electrons in solids that are delocalized over large distances, electron translational energies can be much smaller and the separation of time scales need not necessarily hold.

In recent years there has been a flurry of new work casting doubt on the validity of the Born-Oppenheimer approximation for reactions at metals. These include the failure of the standard model to accurately account for the experimentally observed N_2 vibrational excitation in the recombination of N atoms desorbing from ruthenium (*6*) and in another case the incidence energy dependence of O_2 dissociative adsorption on aluminum (*7, 8*). In our laboratory, we performed experiments showing that hundreds of kilojoules of vibrational energy per mole can be transferred from a "hot molecule" to electrons of a metal; this work culminated in the observation of vibrational promotion of electron emission from a metal with a low work function (*9*, *10*). This result showed explicitly, by direct detection of the hot electron essentially blown off the surface by the force of the molecular vibration, that the Born-Oppenheimer approximation broke down. The topic of Born-Oppenheimer breakdown has become an active forefront area of research in surface chemistry.

Nieto *et al.* show that despite these clear indications of the importance of Born-Oppenheimer breakdown, one is not precluded from using the standard model of reactivity in all cases. In other words, there certainly are some reactions at metal surfaces (perhaps most; time will tell) that are well described by the standard model of reactivity. In this work, comparisons are made between experiment and theory in one of the simplest and best characterized surface chemical reactions, H_2 interacting with a platinum surface. It is noteworthy that Nieto *et al.* are able to use a highly sophisticated version of the standard model, where six degrees of freedom are treated quantum mechanically—a technical tour de force. The 6D quantum approach is essential because H_2 and H exhibit quantum interference (wave behavior) effects as a result of their low masses. When H_2 collides with platinum, it may bounce off and diffract quantum mechanically or dissociate, forming adsorbed H atoms on the surface. The first-principles simulation of H_2 on platinum reported by Nieto *et al*. captures in a nearly quantitative fashion both of these very different kinds of collisional processes. This is a remarkable success for the standard model of reactivity and provides new motivation to seek the limits of this approach, which have not yet been identified clearly.

Future work will certainly focus on helping to better define under what conditions the standard model of reactivity can be applied to catalytically important reactions at metal surfaces. In addition, theorists are actively striving to develop the next generation of chemical simulation packages that can take into account the role of excited electronic states in surface chemistry, going beyond the Born-Oppenheimer approximation. Such developments will make important contributions to our understanding of all kinds of chemistry involving excited electrons in solids. For example, our ability to learn how to power catalytic processes with light (photocatalysis) as opposed to heat (conventional thermal catalysis) will rely on new understanding of excited states in solids, an area of future technology that is essential to a world with diminishing cheap oil reserves.

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DEVELOPMENTAL BIOLOGY

Mixed Messages in Early Development

MicroRNAs, molecules that repress gene expression, fine-tune early embryogenesis. Rather than expressing genetic information supplied in the egg from the mother, microRNAs direct the developing embryo to express its own genome instead.

Stephen M. Cohen and Julius Brennecke

During oogenesis, the egg is loaded with nutrients, proteins, and messenger RNAs (mRNAs) produced in the ovary by the mother. Many of these "maternal mRNAs" encode proteins that are needed for early development of the embryo, before the onset of new mRNA synthesis that is directed by the embryo's own genome. Soon after fertilization of the egg, a transition occurs from use of maternal mRNAs to expression of the zygotic genome (see the figure). On page 75 of this issue, Giraldez and co-workers report that the zebrafish microRNA-430 (miR-430) family contributes to this transition by promoting turnover of maternal mRNAs (*1*).

MicroRNAs (miRNAs) are small noncoding RNAs that serve as posttranscriptional regulators of gene expression (*2*, *3*). They provide sequence information needed to guide ribonucleoprotein complexes (miRNPs) to target mRNAs, leading to repression of their translation and enhanced turnover. The 5′ end of a miRNA, called the seed region, confers much of the target recognition specificity. Computational and experimental studies have shown that miRNAs typically have hundreds of target sites in a given transcriptome, most often located in the 3′ untranslated region (3′ UTR) of a target mRNA (*4*–*7*). Recent studies based on miRNA target prediction and on comparison of miRNA and target mRNA expression suggest that miRNAs may help to reduce expression of mRNAs to inconsequential levels in cells where they are no longer needed or where their expression might be detrimental (*8*, *9*).

The new findings by Giraldez *et al*. provide an elegant example of this principle in action. In previous work (*10*), they identified miR-430 as an abundant early expressed miRNA in the developing zebrafish embryo. Subsequent cloning efforts showed that miR-430 is the only abundant miRNA in the first 4 to 8 hours of development (*11*). miR-430 is encoded by a large gene family (more than 90 members) that produces several, slightly different, forms of the mature miRNA. Because these miRNAs are expressed at the same time and place and share the same seed sequence, they are expected to have largely overlapping sets of targets. Expression of miR-430 begins at the moment of transition from maternal to zygotic gene expression, and mature miR-430 rapidly accumulates to high levels.

In view of the complexity of the miR-430 gene family, generating mutant zebrafish that lack it would be a daunting task. However, because this miRNA is the only one expressed

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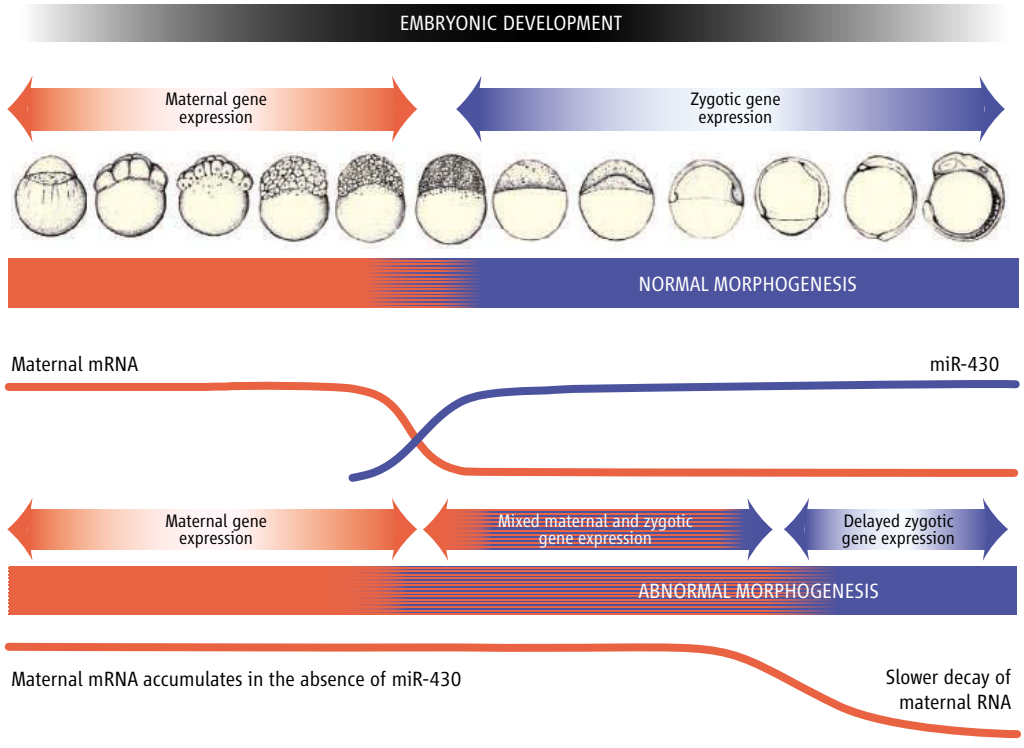
so early in the embryo, the authors were able to apply a clever trick to produce embryos lacking it. The ribonuclease III enzyme Dicer processes miRNA precursors to produce mature miRNAs. Dicer is provided to the egg as a maternal mRNA in amounts sufficient to support embryonic development, but it is also expressed zygotically (*12*). With the use of germ cell transplantation, it is possible to produce adult female fish that have a germline lacking Dicer activity. These fish can then be used to produce "Dicer-free" embryos that are incapable of processing miRNA precursors and, as such, lack mature miRNAs. Dicer-free embryos exhibit subtle defects in gastrulation and brain morphogenesis, most of which can be suppressed by injecting the embryo with mature miR-430 (*10*).

Giraldez *et al*. used this method to assess the effects of miR-430 on mRNA levels in the early zebrafish embryo. By comparing expression profiles of Dicer-free embryos to those of embryos supplemented with miR-430, they estimate that several hundred mRNAs are likely to be direct targets of destruction by miR-430 in the early embryo. Remarkably, about 40% of maternal mRNAs may be affected. This genome-wide analysis is substantiated by extensive experiments showing that regulation of many of the identified targets is posttranscriptional and mediated by their 3′ UTRs, and that a subset of these depend on the presence of the identified miRNA target sites. Thus, the biological effects can be attributed to a direct interaction between miR-430 and the targets.

How does miR-430 control the abundance of these RNAs? A miRNA can induce cleavage of a target mRNA if the degree of sequence complementarity between them is high enough. That, however, is rarely the case in animals. Nonetheless, miRNAs can reduce the level of many mRNAs that contain only imperfect target sites (*13*). Recent work suggests that miRNAs direct their targets to P-bodies, cytoplasmic foci where rapid mRNA decapping and degradation occur (*14*–*16*). Yet the mechanistic link between miRNP binding and target mRNA localization to P-bodies has remained unclear. Giraldez *et al*. add to this picture by showing that miR-430 promotes the rapid deadenylation of target mRNAs. The polyadenosine tail of a mRNA contributes to its stability and enhances mRNA translation into protein. Polyadenylation of maternally deposited mRNAs is an important regulator of their expression in the embryo. Giraldez *et al*. found that target mRNAs were adenylated on schedule but were then rapidly deadenylated, limiting the window for efficient expression. This rapid deadenylation required the presence of miR-430 and its target sites in the 3′ UTR of the regulated mRNAs. Another recent report (*17*) suggests that target mRNA deadenylation is a general consequence of miRNP recruitment. Whether deadenylation is the primary cause of target accumulation in P-bodies or vice versa remains to be determined.

Perhaps the most intriguing outcome of this study is the finding that miR-430 targets maternal mRNAs to promote their turnover. A consequence of removing miR-430 activity is that these mRNAs are not cleared efficiently and continue to be present, and presumably translated into protein, for longer than normal. This situation at least partially blurs the transition from maternal to zygotic control of embryonic development (see the figure). In view of the resulting substantial shift in gene expression, it seems surprising that mixing maternal and zygotic mRNAs does not have more profound consequences for the embryo. Presumably, these maternal mRNAs eventually decay, but they do so more slowly than in the presence of miR-430. This study provides compelling support for the emerging view that many miRNAs fine-tune development to ensure robustness, rather than act as developmental switches (*8*, *9*). Early and abundant expression of miR-430–related miRNAs is conserved among vertebrates, so the function of these miRNAs in the maternal-zygotic transition may be a general feature of vertebrate embryogenesis, even though very different maternal mRNAs may have acquired target sites over time in different species.



Fine-tuning embryonic development. In early zebrafish embryogenesis, the microRNA miR-430 regulates the transition from maternal to zygotic mRNA transcription by targeting maternal mRNAs for degradation. In the absence of miR-430, maternal mRNAs accumulate and interfere with morphogenesis. [Adapted from (*10*)]

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C–H Bond Functionalization in Complex Organic Synthesis

Kamil Godula and Dalibor Sames*

Direct and selective replacement of carbon-hydrogen bonds with new bonds (such as C–C, C–O, and C–N) represents an important and long-standing goal in chemistry. These transformations have broad potential in synthesis because C–H bonds are ubiquitous in organic substances. At the same time, achieving selectivity among many different C–H bonds remains a challenge. Here, we focus on the functionalization of C–H bonds in complex organic substrates catalyzed by transition metal catalysts. We outline the key concepts and approaches aimed at achieving selectivity in complex settings and discuss the impact these reactions have on synthetic planning and strategy in organic synthesis.

Organic compounds consist of chains or rings of consecutive carbon atoms, each capped with one or more hydrogen atoms. This scaffolding, interrupted and adorned with occasional "heteroatoms" (mainly oxygen, nitrogen, phosphorus, sulfur, and the halogens), underlies the extraordinary array of small molecules and biopolymers that comprise living organisms as well as such diverse materials as crude petroleum, pharmaceuticals, molecular switches, and plastics (Fig. 1).

Organic synthesis relies on the transformation of functional groups, or structural features exhibiting relatively high chemical reactivity. C–H bonds are not generally viewed as functional groups in this context. Thus, installment of a new bond requires the presence of either a heteroatom, such as oxygen or a halogen, or unsaturation (i.e., absence of hydrogens) in the carbon backbone (Fig. 2). This logic underpins the process of synthetic planning or synthetic strategy (*1*). The reactive sites or functional groups are typically incorporated by means of multiple transformations; consequently, the starting materials are often rather dissimilar from the final products. This is illustrated by the sequence of several steps that converts compound **1** to product **2** (Fig. 3).

In this light, it becomes clear that the introduction of new functionality directly through transformation of C–H bonds unlocks opportunities for markedly different synthetic strategies. For example, the same target molecule (**2** in Fig. 3) may be accessed in a single step by displacement of a hydrogen atom. Considering the high abundance of C–H bonds, precise one-step substitution of carbon-hydrogen bonds with C–C or C–X bonds (where X is O or N), without disruption of the surrounding molecular structure, carries considerable appeal for synthesis. Thus, selective C–H bond functionalization, as exemplified by the direct conversion of compound **3** to product **2** (Fig. 3), provides straightforward and concise approaches where the topology, or the overall skeletal structure, of the starting material resembles that of the product ("topologically obvious assembly").

In addition to the assembly of specific target molecules, C–H bond functionalization also reshapes synthetic strategies for preparation of series of compounds ["structural core diversification" (Fig. 4)]. The ability to selectively target a number of different C–H bonds in a complex substrate permits direct access to multiple analogs from a common structural predecessor. This sharply contrasts with traditional approaches, wherein multistep, and often distinct, de novo sequences are required for each derivative.

Thus, by viewing C–H bonds as "ubiquitous functionality," we are opening a new chapter in organic synthesis with many exciting opportunities. Advances in homogeneous transition metal catalysis have identified a number of new transformations of C–H bonds, and the strides made in elaborating simple hydrocarbons have been amply reviewed elsewhere (*2*, *3*). Here, we highlight C–H bond functionalization in the context of complex organic molecules—which contain many different kinds of C–H bonds, as well as reactive functionalities—and outline the key approaches leading to selective functionalization. We also discuss the impact of these reactions on synthetic planning and strategy in organic synthesis.

Radical Beginnings: Intramolecular Radical Reactions

Early approaches to functionalization of isolated alkyl C–H bonds (unactivated sp^3 bonds) relied on highly reactive intermediates, including free oxygen and nitrogen radicals. In complex substrates, regioselectivity was achieved by exploiting structural proximity between the high-energy radical, generated transiently in the reaction mixture, and the alkyl group resulting in the intramolecular hydrogen atom abstraction (Fig. 5). The long history of these reactions dates back to the studies carried out by Hoffmann in the late 1800s (*4*), showing that homolysis of bromamines and chloramines led to functionalization of δ-methylene or methyl groups. The synthetic possibilities presented by this process, known today as the Hoffmann-Löffler-Freytag reaction, were realized in the synthesis of nicotine (*5*) and, much later, in the synthesis of conanine steroidal alkaloids (Fig. 5) (*6*, *7*). Analogous pro-

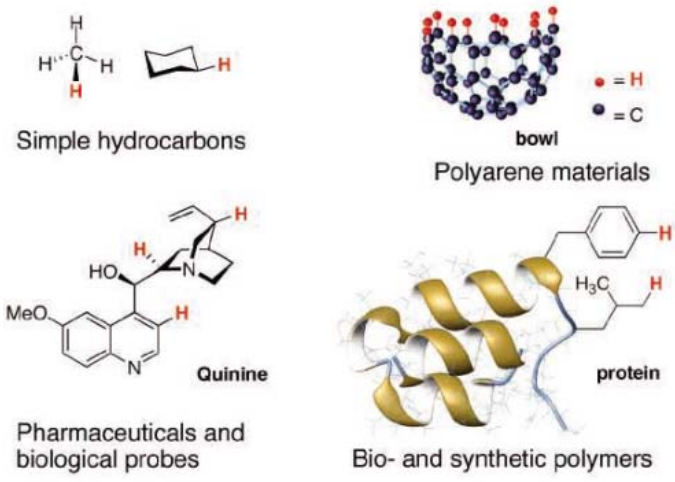

Fig. 1. C–H bonds are found in nearly all organic compounds. C–H bond functionalization will influence the broad field of chemical synthesis. Hydrogen atoms in red designate examples of different C–H bonds in diverse organic compounds.

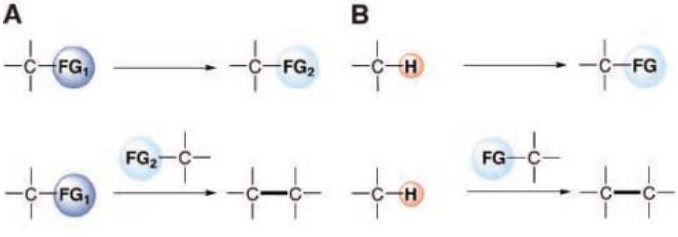

Fig. 2. (**A**) Traditional approach to organic synthesis by means of functional group (FG) transformation. (**B**) Synthesis by means of C–H bond functionalization.

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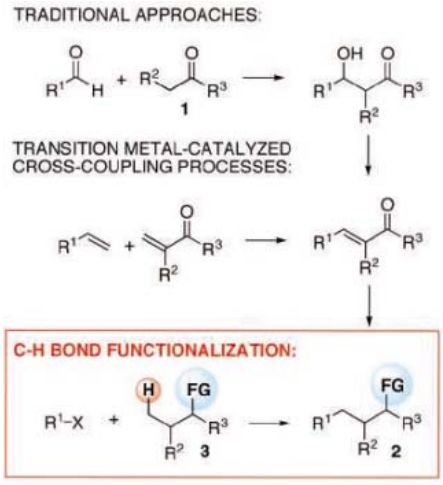

Fig. 3. Evolving algorithms in organic synthesis. R, alkyl or aryl group.

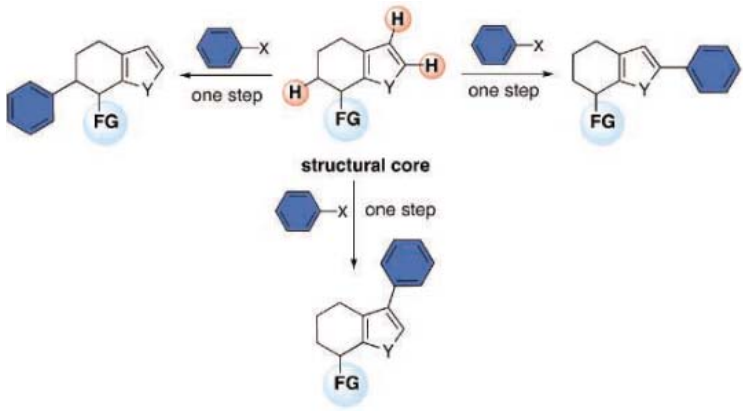

Fig. 4. Structural core diversification by means of C–H bond functionalization: Systematic functionalization of complex motifs provides direct access to a series of structural analogs.

cesses initiated by an oxygen-centered radical were also developed (*8*). Most notably, Barton and Beaton introduced the photolysis of a nitrite ester as a means for converting an isolated methyl group into an oxime in one step. This transformation formed the key step in the synthesis of aldosterone acetate from readily available corticosterone acetate (*9*). Despite its low yield, this synthesis was a landmark achievement in demonstrating the potential of C–H functionalization in the context of an important problem.

These early examples also show that, in a general sense, the functionalization of unactivated sp^3 bonds results in profound strategic advantages; the alternative methods include either a stepwise functional group shuffle (from an existing functional group to the distant unactivated position) or de novo synthesis, neither of which can match the efficiency of the direct functionalization process.

Intramolecular Transition Metal–Catalyzed Carbene and Nitrene Insertion

Analogous to radicals, carbenes can also serve as reactive intermediates for C–H functionalization. In contrast to free carbenes, the corresponding transition metal carbenoids readily available by decomposition of diazocarbonyl substrates offer more control over the reaction course. In particular, the introduction of $Rh_2(OAc)_4$ as a versatile catalyst led to the development of a powerful synthetic methodology with a wide substrate scope. The rhodium-dimer is thought to chaperone the carbene insertion into the C–H bond in a direct fashion, without forming a new C–M intermediate (Fig. 6, top) (*10*). Thus, readily available diazocarbonyl substrates can be converted in one step to cyclic ketones, lactones, and lactams by means of regioselective C–C bond formation at the alkyl site. A wide variety of C–H bonds can be functionalized in this manner, including sterically hindered sp^3 C–H bonds. Furthermore, an excellent level of stereo- and enantioselectivity can be achieved in many different structural contexts by the proper choice of a chiral dirhodium catalyst (Fig. 6A) (*11*).

These reactions are frequently applied to the synthesis of advanced intermediates and natural products (*12*). Several features make them attractive in this respect, including neutral reaction conditions, good functional group tolerance, and a high degree of stereoselectivity. They provide a unique and direct strategy for preparation of valuable cyclic products, one that is orthogonal to the alternative multistep routes.

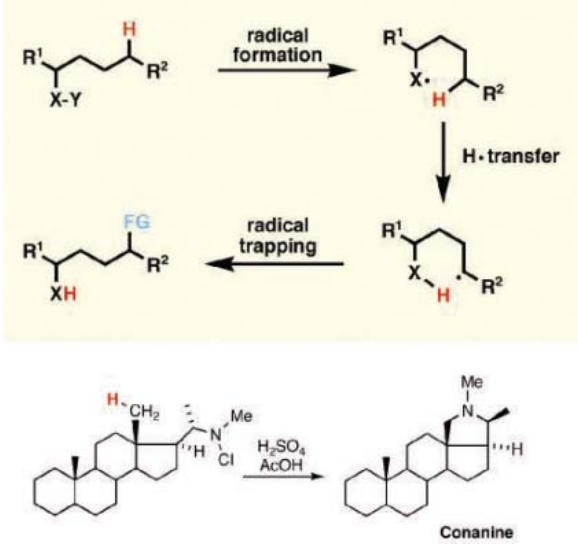

Fig. 5. Intramolecular radical reactions. Hydrogen atoms in red are those substituted in the depicted process. Me, methyl group.

C–N bond formation at an isolated alkyl site, achieved by transition metal–catalyzed nitrene insertion, was pioneered by Breslow (*13*) and subsequently developed into an attractive methodology by Du Bois (*14*). Although the detailed mechanism is a subject of debate, it can formally be viewed as a nitrene insertion, a process analogous to the carbene relative. Cyclization of readily available carbamate or sulfamate substrates was achieved with the use of a $Rh_2(OAc)_4$ catalyst, in the presence of a $PhI(OAc)_2$ oxidant and a MgO base (Fig. 6B). This mild process is regio- and stereoselective, allowing for the introduction of a nitrogen atom at a late stage of the assembly. The strategic advantages of both carbene and nitrene insertion reactions have recently been demonstrated in a highly complex context of an elegant synthesis of natural product tetrodotoxin (Fig. 6C) (*15*).

C–H Functionalization by Means of Coordination-Directed Metallation

Directed metallation is another powerful approach for selective functionalization of C–H bonds in complex substrates applicable to a variety of different C–H bonds (sp^2 and sp^3), including those of isolated alkyls. It entails the use of a suitable heteroatomic function in the substrate to direct a metal complex to the vicinity of a distant C–H bond. The resulting metallacycle, usually a five- or six-membered ring, serves as a versatile intermediate en route to products containing new C–C or C–X bonds (Fig. 7).

In terms of structural complexity, one of the most advanced examples of directed metallation was carried out in the context of the synthesis of the antitumor alkaloid rhazinilam (*16*). The pivotal step involved selective dehydrogenation of the diethyl segment in intermediate **4**. This was achieved by the attachment of a platinum complex to the aniline nitrogen to form complex **5** and subsequently the reactive intermediate **6** (Fig. 7A). Thermolysis of complex **6** provided platinum hydride **7** as the major product through a sequence of C–H bond ac-

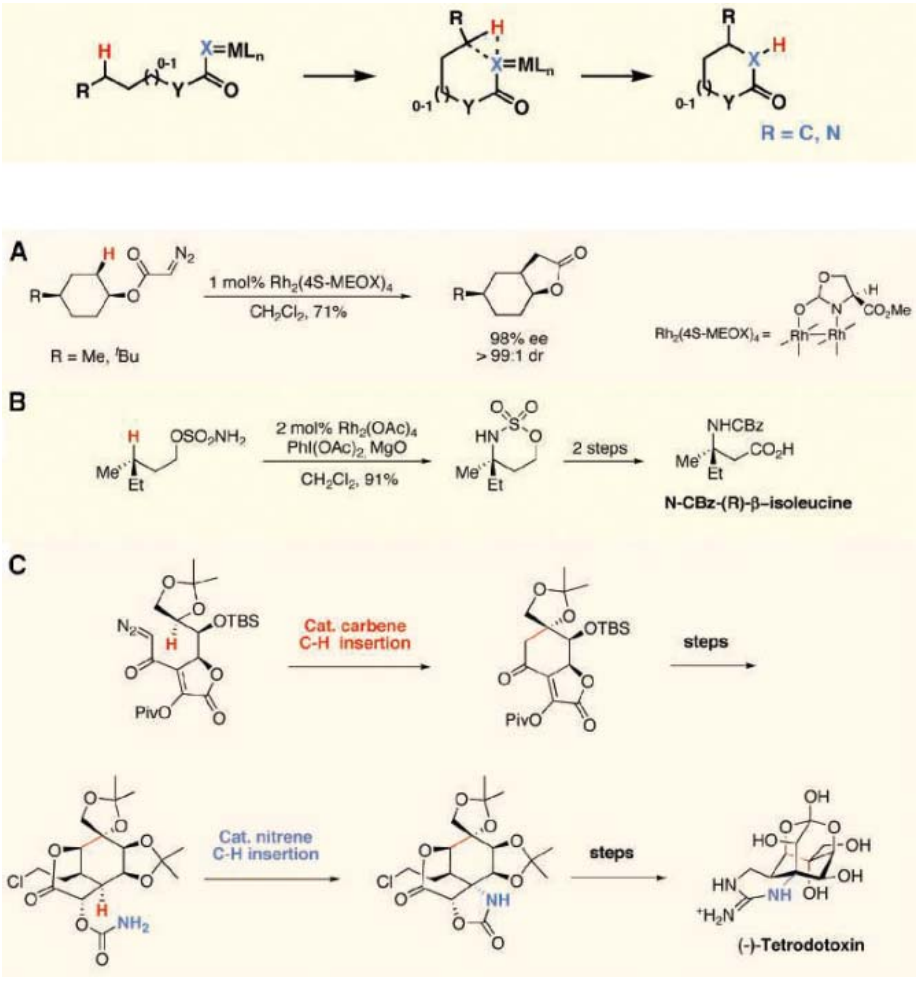

Fig. 6. (**A** to **C**) Intramolecular carbene and nitrene insertion into sp^3 C–H bonds. ML_n, general representation of a transition metal complex with a number (*n*) of ligands (L); ^{t}Bu, *tert*-butyl group; TBS, *tert*-butyldimethylsilyl group; Piv, pivaloyl group; CBz, benzyl carbamate; ee, enantiomeric excess; dr, diastereomeric ratio; (-) designates a levorotatory enantiomer.

tivation and β-hydride elimination. Notably, selective functionalization was achieved at the least reactive site, namely the isolated ethyl group, in the presence of notoriously reactive pyrrole and aniline rings.

Although this process was stoichiometric in platinum, it demonstrated the potential of C–H functionalization in synthesis of natural products and at the same time indicated that the transition metal chemistry developed for simple hydrocarbons [i.e., methane activation by Shilov chemistry (*17*)] was applicable to complex organic substrates. On a strategic level, it showed how C–H functionalization can give rise to novel and direct strategies for synthesis of complex natural products.

The versatility of palladium chemistry can be harnessed by both formation and utilization of palladacycles (*18*). Synthesis of the alkaloid teleocidine inspired the development of new C–C bond formation by means of transmetallation of palladacycle **8** with aryl- and alkenylboronic acids (*19*). Although the substrate scope is rather limited, this process represents a prototype for a direct arylation or alkenylation of unactivated sp^3 C–H bonds (Fig. 7B).

The directed platination and palladation approaches have also been used for selective oxygenation of isolated alkyl groups. Catalytic hydroxylation of α amino acids has been reported (*20*). For example, L-valine was converted to γ-lactone **9** in water in the presence of K_2PtCl_4 catalyst [1 to 10 mole percent (mol %)] and $CuCl_2$ oxidant (Fig. 7C). A number of substrates may be hydroxylated, including norvaline, leucine, isoleucine, *n*-butylamine, and valeric acid. Mechanistic study showed that the Pt(II) center cleaved the C–H bond, yielding a putative platinacycle intermediate, which underwent fast oxidation to a platinum (IV) intermediate, which in turn collapsed to the lactone and the platinum(II) catalyst. Thus, the direct functionalization of natural amino acids afforded valuable intermediates (γ-lactones) in one step without the use of organic solvents. Rooted in the Shilov platinum chemistry, this process represents an early example of selective hydroxylation of complex substrates by a simple transition metal catalyst. It differs conceptually from methods based on free radical chemistry (e.g., Fenton chemistry—oxidation with free hydroxyl radicals) or metal-oxo chemistry (P450 enzymes or small molecule mimics) (*21*).

Cyclopalladation of oximes has recently been re-examined and has formed the basis for a new catalytic process. Aliphatic oximes can undergo acetoxylation of the α methyl group catalyzed by $Pd(OAc)_2$ in the presence of $PhI(OAc)_2$ as the oxidant (Fig. 7D) (*22*). A minor alteration of the oxidant system also allows for formation of C–I bonds, as recently demonstrated in diastereoselective iodination of a methyl group under the direction of chiral oxazoline auxiliary (*23*). This is an active area of research that will continue to generate new catalytic protocols for both C–X and C–C bond formation directed by a variety of functional groups.

Coordination-directed metallation has also been the underlying mechanism for selective C–C formation at arene rings (sp^2 C–H bonds) in the ortho position to a suitable functional group. The key discovery in this area was made in the Murai group (*24*), which demonstrated the first efficient, catalytic, and selective coupling of an arene C–H bond and an alkene. Aromatic ketones may be alkylated exclusively in the ortho positions in the presence of a ruthenium catalyst under neutral conditions (Fig. 8A). This report attracted much interest and inspired the development of related reactions (*25*).

The likely mechanistic scenario involves the insertion of the low-valent ruthenium to the ortho-arene C–H bond affording a metallacycle intermediate (**10** in Fig. 8), which facilitates the subsequent C–C bond formation affording the product and the regenerated catalyst. The overall process provides a neutral alkylation method catalytic in the transition metal.

Substrates other than aromatic ketones, namely enones (*26*) and α,β-unsaturated esters (*27*), can be alkylated at the β position, whereas aldehydes can be converted to ketones by means of the insertion of alkenes into aldimine C–H bonds (*28*). In addition to alkylation, ortho-acylation occurs when a mixture of alkene and carbon monoxide is used (*29*). It was shown that the sp^2 nitrogen of imines and pyridines may also serve as the directing group, usually under the action of rhodium (I) complexes. Recently, Bergman and Ellman have reported the rhodium-catalyzed intramolecular alkylation of ketimines, affording new annulation methodologies for the formation of five- and six-membered cycles (Fig. 8B). This methodology allowed for straightforward and efficient access to a biologically active tricyclic analog of mescaline (*30*). A high degree of asymmetric control was also achieved in the presence of a chiral ligand (*31*).

The catalytic approach differs from traditional protocols on both a mechanistic and an operational level. For example, ortho-lithiation,

although a highly useful method for elaboration of arenes, requires a stoichiometric amount of strong base (butyl lithium), which has certain limitations with regard to the choice of suitable directing groups. It may also be problematic for substrates with sensitive functional groups. Similarly, electrophilic approaches do not provide access to the same products because the reactivity and regioselectivity preferences of Friedel-Crafts alkylation (electrophilic aromatic alkylation), dictated by the reaction mechanism, are completely different from those of the directed catalytic methods discussed above. For instance, product **11** shown in Fig. 8 would not be obtained under Friedel-Crafts conditions. Thus, the catalytic approach not only represents milder alternatives to standard ionic protocols but also unlocks a new scope of products inaccessible through the standard chemistries.

Catalytic ortho-arylation has also been developed for a variety of substrates, including aromatic imines, 2-aryl-oxazolines, -imidazolines, and -pyridines. Readily available haloarenes serve as arene donors in the presence of the ruthenium catalyst and a weak base (Fig. 8C) (*32*). The synthetic appeal of this process is readily apparent; the contiguous substitution patterns around the aromatic ring (e.g., 1,2,3-trisubtituted benzene derivative **12**) can be accessed in a highly efficient manner. Direct C–H arylation reactions offer a distinct advantage over standard cross-coupling reactions [e.g., Suzuki coupling (*33*)] in that they eliminate the need for functionalization of the substrates (such as the formation of a halide or a boronate ester) before C–C coupling. This seemingly small point carries important consequences in the synthesis of heavily substituted biaryl compounds.

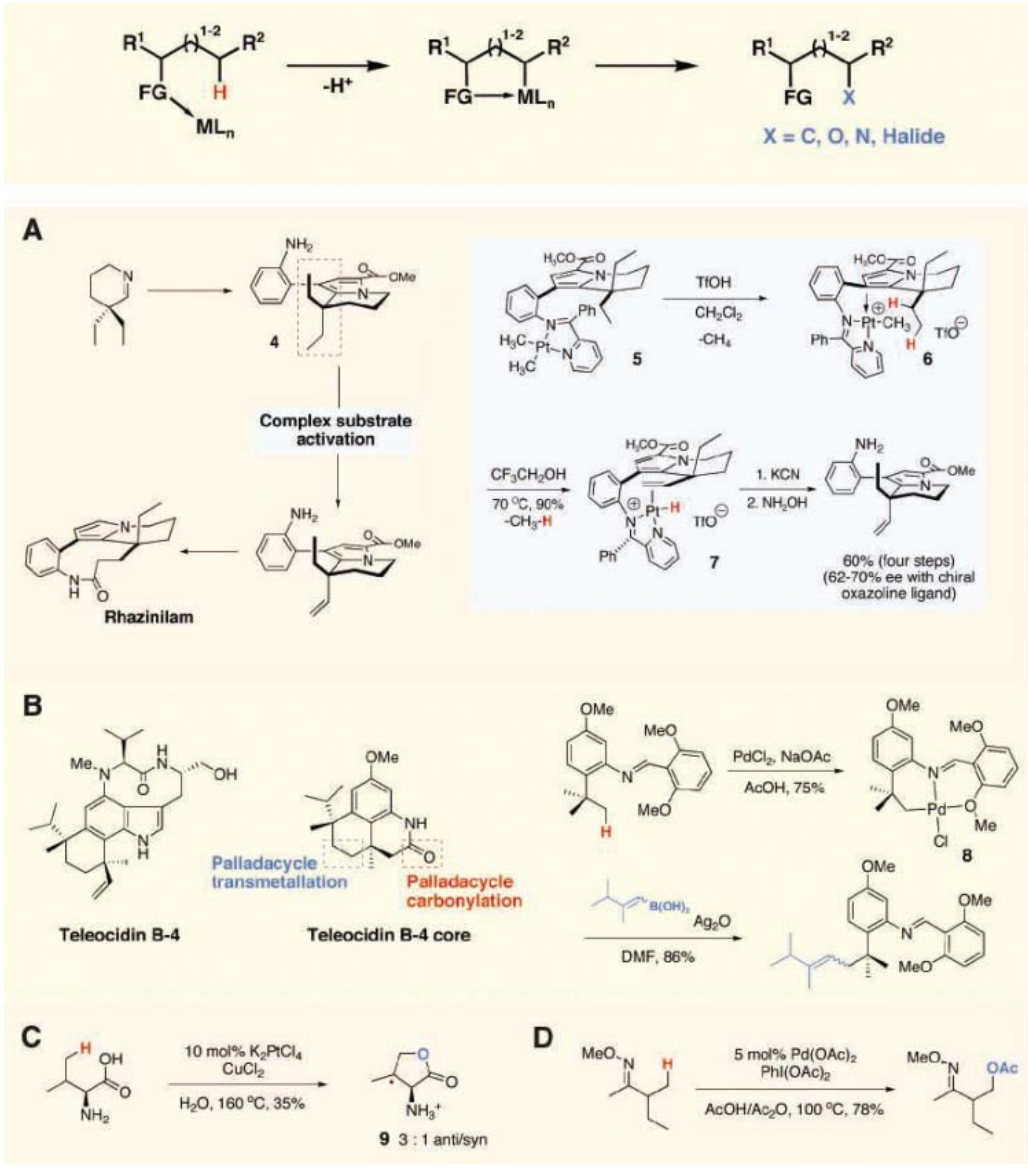

Fig. 7. (**A** to **D**) Functionalization of sp^3 C–H bonds by means of coordination-directed metallation. TfOH, trifluoromethanesulfonic acid; AcOH, acetic acid; Ac_2O, acetic anhydride; DMF, dimethylformamide; syn/anti, ratio of isomers with a pair of substituents on the same face (syn) and the opposite face (anti) of the lactone ring.

Directed metallation is a powerful approach that enables regioselective functionalization of C–H bonds in diverse structural contexts ranging from isolated alkyl groups to aromatic rings. The transition metal in cooperation with the directing group cleaves the targeted C–H bond, and the corresponding metallacycle serves as a versatile intermediate for either C–C or C–X bond forming processes. The availability of a variety of suitable metals—including electrophilic palladium and platinum salts or low-valent ruthenium, rhodium, and iridium complexes—lends sufficient flexibility for reaction design and development. This is an active area that will continue to generate new catalytic and selective C–H functionalization methods.

Other Approaches to Selective C–H Bond Functionalization

Aside from the intramolecular and directed methods, what other concepts and approaches may afford intermolecular and selective functionalization of C–H bonds in complex substrates? Can we generate relatively simple catalysts with programmable selectivities?

One such approach would require the catalyst to discern relative strength and stereoelectronic properties of C–H bonds. For example, sp^3 C–H bonds adjacent to heteroatoms, typically oxygen or nitrogen (α position of ether or amine) or an arene ring (benzylic position), are referred to as "activated," owing to their lower thermodynamic strength and higher reactivity in comparison to isolated alkyl C–H bonds.

For example, intermolecular carbene insertion reactions have been reported in neat hydrocarbons (*34*); however, in complex substrates, insertion occurs at activated positions. Davies has shown impressively broad scope for this reaction class, albeit with some limitation on the carbene precursor (*35*). Namely, α-aryl- and α-vinyl-diazocarbonyl compounds are required to disfavor carbene dimerization relative to C–H insertion. Otherwise, this method can substitute the α positions of ethers, amines, and carbamates, as well as benzylic and allylic positions. In a crude sense, the regioselectivity seems to be determined primarily by the C–H bond strength and secondarily by the steric hindrance (Fig. 9).

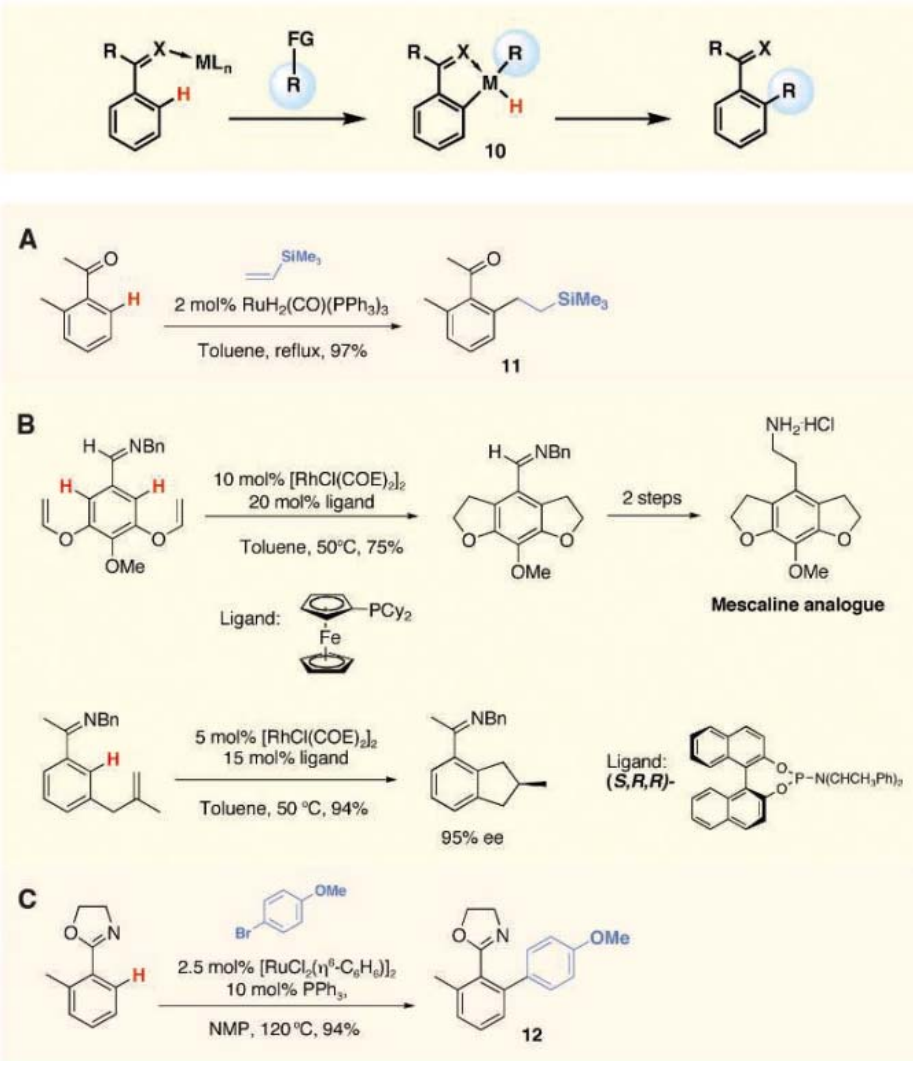

Fig. 8. (**A** to **C**) Functionalization of arene C–H bonds by means of coordination-directed metallation. Cy, cyclohexyl group; Bn, benzyl group; COE, cyclooctene; NMP, *N*-methylpyrrolidinone; PPh_3, triphenyl phosphine.

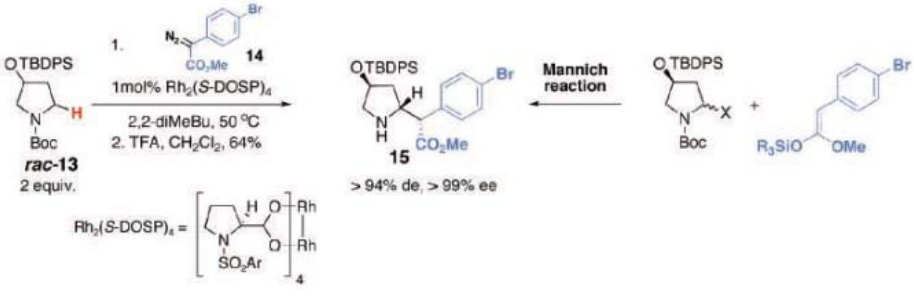

Fig. 9. Enantioselective intermolecular carbene insertion into activated sp^3 C–H bonds. R, alkyl group; TBDPS, *tert*-butyldiphenylsilyl group; Boc, *tert*-butyl carbamate; X, halide; de, diastereomeric excess.

Furthermore, the chiral dirhodium catalysts allow for excellent control of stereo- and enantioselectivity. This could be demonstrated by the kinetic resolution of a racemic pyrrolidine substrate **rac-13**. Coupling of **rac-13** with diazophenylacetate **14** afforded compound **15** as the major product with high diastereo- and enantioselectivity (Fig. 9) (*36*). This one-step process represents a highly attractive alternative to the traditional Mannich reaction (*37*). For the latter process, a regioselective oxidation of the corresponding pyrrolidine would be required before the C–C bond formation. Thus, carbene insertion with protected amines affords Mannich products; similarly, silylethers can directly lead to aldol products under neutral conditions. This method enabled efficient syntheses of pharmaceuticals and natural products.

The next key question in this context is whether a programmable selectivity can be achieved among unactivated sp^3 C–H bonds—i.e., within a long isolated alkyl group—in the absence of the suitable directing mechanisms discussed earlier, by the actions of a small transition metal catalyst. A recent report described selective borylation of terminal methyl groups in substrates containing a number of functional groups including ether, ketal, amine, and fluoride (Fig. 10) (*38*). It appears that the high selectivity is primarily governed by the sensitivity of the rhodium catalyst to steric hindrance. Furthermore, in cases where multiple methyl groups are present, a preference for the least electron-rich C–H bonds was observed. It is notable that a small metal catalyst can achieve such a high level of reactivity and selectivity, highlighting the potential of transition metal chemistry.

Selective targeting of internal isolated sp^3 C–H bonds in complex targets has hitherto been limited to enzymatic or biomimetic systems (*39*) equipped with a recognition element capable of arranging the C–H bond of interest and the reactive center in a proximal and favorable orientation. At present, it seems that the reactivity differences between isolated alkyl C–H bonds (e.g., two adjacent methylenes in the middle of a long alkyl group) are very small to tackle this problem in the absence of the molecular recognition element. For now, this general goal represents the ultimate chemo-, regio-, and stereoselectivity to be achieved and continues to serve as an inspiration for the future generations of scientists.

In the area of aromatic systems, the unique properties of transition metal catalysts may lead to regioselectivity preferences that are complementary to the standard ionic methods. A novel process for catalytic borylation of aromatics with pinacolborane or pinacoldiboron has recently been introduced. Two catalytic systems have been developed for this transformation, namely iridium (I) phosphine complexes (*40*) and iridium (I) bipyridyl complexes (Fig. 10) (*41*). Both methods exhibit an impressive substrate scope, including electron-rich and electron-poor arenes and heteroarenes. Regioselectivity at the benzene ring is strictly controlled by sterics, placing the boronate in the least hindered position. Hence, these methods not only allow for one-step preparation of areneboronates, but also afford isomers that are not readily available by means of halogenation or ortho-lithiation. The observed kinetic trends (faster rates with electron-deficient arenes) imply that the key step may involve either oxidative addition of the C–H bond to the iridium metal or σ-bond metathesis between the C–H bond and Ir–B bond.

Because heteroaromatics are indispensable core structures of pharmaceuticals, biological

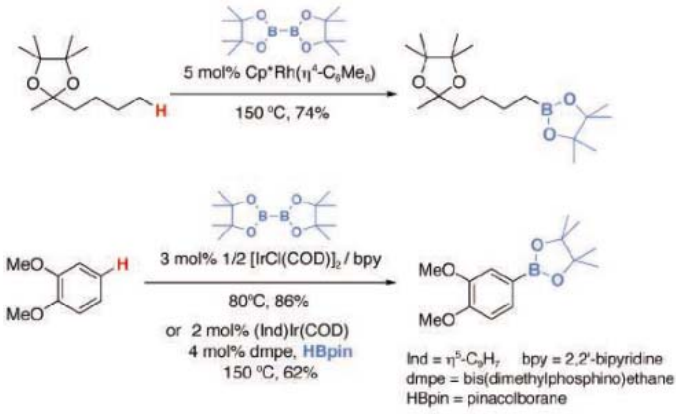

Fig. 10. Borylation of alkyl and aryl C–H bonds. COD, cyclooctadiene.

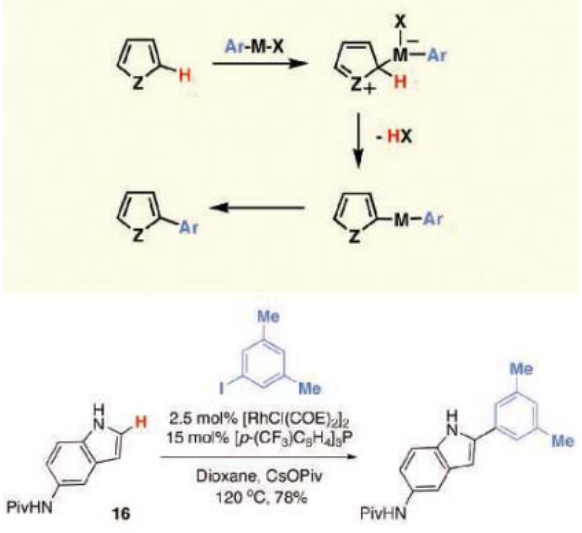

Fig. 11. Arylation of heteroarene C–H bonds. M, transition metal; Z, nitrogen, oxygen, or sulfur atom; Ar, aryl group; CsOPiv, cesium pivalate.

probes, and other functional synthetics, considerable attention is currently focused on catalytic functionalization of these compounds. After the early reports, several laboratories have been engaged in the development of new methods for arylation of electron-rich heteroarenes, such as furans, thiophenes, and (NR)-azoles (*42*–*44*). These protocols usually rely on a palladium catalyst in the presence of a weak base. Arylation of (NH)-azoles in a free form has been demonstrated. Fig. 11 illustrates an example of rhodium-catalyzed arylation of indole **16** (*45*). Notably, this catalytic system targets the C–H bond at the 2 position in the presence of two N–H bonds of relatively high acidity.

The experimental evidence suggests that these reactions proceed by means of an electrophilic metallation mechanism. However, in contrast to standard electrophiles (e.g., halogens and acyl halides), the transition metal catalyst prefers the cleavage of the C–H bond adjacent to the nitrogen atom, which in turn determines the final site of arylation. An alternative mechanistic possibility involves a Heck-type process. Although Heck coupling—which entails catalytic coupling between haloarene and alkene—may also be classified as catalytic C–H functionalization transformation, this important process has been extensively studied and reviewed (*46*).

Arylation of electron-deficient positions of heteroaromatic compounds has also been demonstrated, although with limited scope and efficiency (*47*–*49*). However, this is an active area of research, and advances in terms of substrate scope, catalyst efficiency, and operational issues may be expected in the coming years.

Conclusions

C–H bond functionalization is an area of rapid growth that will continue to push the limits of chemical reactivity to the extent that C–H bonds may soon be viewed as ubiquitous functional groups (*50*). Such an ability to transform a variety of C–H bonds unlocks entirely new perspectives in complex organic synthesis. The direct strategies for assembling molecules are readily apparent from a topological perspective and should lead to major simplification of synthetic sequences. Unlocking the reactivity of ubiquitous bonds will also affect the process of molecular design in many diverse areas of research, because novel synthetic processes not only affect how we make desirable materials but also what we make and study.

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Sexual Conflict via Maternal-Effect Genes in ZW Species

Paige M. Miller,[1]* Sergey Gavrilets,[2] William R. Rice[1]

The placenta of eutherian mammals and the endosperm of plants provide an expansive realm for genomic conflict through genetic imprinting (*1*, *2*). Here we show that maternal products in the egg can provide a parallel arena for genetic conflict. The distinction between species in which the heterogamous sex is male (XY) versus female (ZW) has far-reaching consequences for sexual conflict via maternal-effect genes, the products of which can have opposing fitness effects because of the unique developmental trajectories of sons and daughters (Fig. 1). Throughout, the term sex chromosomes refers to their nonrecombining regions in the heterogametic sex.

Maternal effects might lead to sexual conflict in two ways: (i) Incidental harm. Maternal-effect alleles that favor sons but harm daughters through chance pleiotropy accumulate on the Z, and those favoring daughters and harming sons accumulate on the W. (ii) Actively selected harm. When sibling competition occurs, maternal-effect alleles are selected to kill or incapacitate the nontransmitting sex. Z alleles harm daughters and W alleles harm sons [see (*3*) for autosomal examples in the context of selfish elements].

More explicitly, consider a large, randomly mating population with discrete, nonoverlapping generations subject to constant selection. Individual fitness is determined maternally (*4*, *5*). Fitness is defined as viability, mating success (the probability that an adult enters the mating pool), or fertility (a contribution of an individual to the number of offspring produced by the mating pair, assuming that male and female contributions interact multiplicatively). Assume that there are two W-linked alleles: W_0 and W_1. Let Z denote the Z chromosome, and let offspring of ZW_0 mothers have fitness 1. The fitness of daughters and sons of ZW_1 mothers have fitness $1 + B_{\text{daughter}}$ and $1 - C_{\text{sons}}$, respectively; i.e., ZW_1 maternal effects benefit their daughters at a cost to their sons. Let p be the frequency of ZW_1 females in the mating pool. Then, it is straightforward to show that the value of p in the next generation is

$$p' = p + \frac{B_{\text{daughters}}\, p(1 - p)}{1 + pB_{\text{daughters}}}$$

From this equation, one concludes that if $B_{\text{daughters}} > 0$ (that is, daughters of ZW_1 mothers get some fitness advantage relative to daughters of ZW_0 mothers), then allele W_1 invades and gets fixed ($p \to 1$) independently of its effect on sons.

Next assume that there are two Z-linked alleles, Z_0 and Z_1. Let W denote the W chromosome and set the fitness of offspring from Z_0W mothers to be 1. Daughters and sons of Z_1W mothers have fitness $1 - C_{\text{daughters}}$ and $1 + B_{\text{sons}}$, respectively. This fitness scheme implies that Z_1W maternal effects benefit sons at a cost to daughters. Let the frequencies of Z_1W females and of allele Z_1 in males in the mating pool be p_{female} and p_{male}, respectively. Then, it is straightforward to show that in the next generation

$$p'_{\text{females}} = p_{\text{males}} \qquad \text{and}$$

$$p'_{\text{males}} = \frac{p_{\text{males}} + p_{\text{females}}}{2} + B_{\text{sons}} \frac{p_{\text{females}}(1 - p_{\text{females}})}{2(1 + B_{\text{sons}} p_{\text{females}})}$$

This system of equations has only two equilibria: $p_{\text{male}} = p_{\text{female}} = 0$ and $p_{\text{male}} = p_{\text{female}} = 1$. If $B_{\text{sons}} > 0$, the former is unstable whereas the latter is stable. This shows that if sons of Z_1W mothers get a fitness advantage, allele Z_1 invades and gets fixed (so that $p_{\text{male}} \to 1$, $p_{\text{female}} \to 1$) independently of its effect on daughters' fitness.

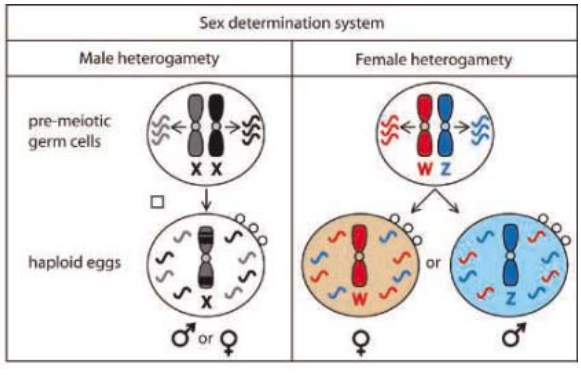

Fig. 1. Maternal-effect alleles on the two X chromosomes (gray/black chromosomes) are transmitted symmetrically to sons and daughters, but in ZW species, the transmission is asymmetrical—Z-linked (blue chromosome) to sons and W-linked (red chromosome) to daughters. Wavy lines depict maternal products coded by the sex chromosomes and packaged in the egg. Blue (sons) and red (daughters) shading of eggs depicts gender-specific differences during subsequent ontogeny of the embryo.

The accumulation of sexually antagonistic maternal-effect genes in ZW species creates the opportunity for an evolutionary arms race. As sexually antagonistic male-benefit maternal-effect alleles accumulate on the Z, and female-benefit maternal-effect alleles on the W, there will be compensatory selection to counteract the harm at genes located on both the other sex chromosome and on the autosomes. Because primitive Z and W chromosomes can carry hundreds of genes, the initial potential for an arms race may be substantial. While the W chromosome decays due to its lack of recombination (*6*), the influence of this chromosome on maternal-effect conflict may diminish. For example, the Z chromosome of the jungle fowl still has at least 516 genes, whereas the W has only 55 (*7*).

Sex-specific gene expression, ontogeny, and physiology provide diverse and simple mechanisms for single-locus maternal-effect genes to harm the sex that is not carrying a maternal Z or W chromosome. The potential for maternal-effect conflict imparts an important distinction between male and female heterogamy. It replaces sexual selection as a source of chronic adaptive evolution that can drive the decay of the nonrecombining W via genetic hitchhiking (*8*). It also increases the scope for genetic conflict in ZW compared with XY species, because the W, unlike the Y, is expressed in both sexes via maternal products in the egg. Species with ZW versus XY sex determination clearly have at least one unique force influencing the evolution of their sex chromosomes.

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Zebrafish MiR-430 Promotes Deadenylation and Clearance of Maternal mRNAs

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MicroRNAs (miRNAs) comprise 1 to 3% of all vertebrate genes, but their in vivo functions and mechanisms of action remain largely unknown. Zebrafish miR-430 is expressed at the onset of zygotic transcription and regulates morphogenesis during early development. By using a microarray approach and in vivo target validation, we find that miR-430 directly regulates several hundred target messenger RNA molecules (mRNAs). Most targets are maternally expressed mRNAs that accumulate in the absence of miR-430. We also show that miR-430 accelerates the deadenylation of target mRNAs. These results suggest that miR-430 facilitates the deadenylation and clearance of maternal mRNAs during early embryogenesis.

MicroRNAs (miRNAs) are small RNA molecules that base pair with target mRNAs to induce posttranscriptional gene repression (*1*, *2*). MiRNAs have important roles during animal development. Interfering with miRNA processing by using mutants for the ribonuclease (RNase) III enzyme Dicer affects morphogenesis during zebrafish embryogenesis and mouse limb development (*3*, *4*). Modulating the function of individual miRNAs leads to defects that range from aberrant cell fate specification and cell death to abnormal cell function (*1*, *2*, *5*, *6*). Despite this progress, it is still poorly understood how miRNAs regulate developmental processes. In particular, the in vivo targets of miRNAs are largely unknown.

Several strategies have been used to identify miRNA targets (*7*). Genetic approaches have identified several miRNA-mRNA target pairs that play important roles during *Caenorhabditis elegans* development (*1*, *2*, *5*, *6*, *8*–*10*). This approach preferentially identifies targets whose reduced activity suppresses miRNA loss-of-function phenotypes. In silico target predictions based on conserved complementarity between miRNAs and orthologous mRNAs have identified ~100 to 200 putative targets for a given miRNA (*7*, *11*–*18*). It is unknown how many true but nonconserved targets are missed in this approach. Cell culture studies have used microarrays to compare mRNA levels in the presence or absence of a transfected miRNA (*19*). This method has led to the identification of ~100 putative targets for miR-1 and miR-124. It is unclear how many of the in silico or cell culture targets are regulated in vivo. Hence, developing an in vivo system to identify a large fraction of biologically relevant miRNA targets could provide major insights into the roles of miRNAs during development.

Zebrafish embryos deficient for maternal and zygotic Dicer activity (MZ*dicer*) cannot generate mature miRNAs (*3*). These mutants display defects during gastrulation and brain morphogenesis. These defects are rescued by injection of processed miRNAs belonging to the miR-430 family. MiR-430 is the most abundant miRNA family expressed during early zebrafish development (*3*, *20*), is conserved in other vertebrates (miR-302 and miR-372) (*3*), and accumulates during the maternal-to-zygotic transition. This transition is a universal process in animal development, when the embryo activates zygotic gene expression and thus no longer solely relies on maternally provided transcripts (*21*). The activation of zygotic transcription coincides with the elimination of many maternal mRNAs by unknown mechanisms. The results presented here indicate that miR-430 accelerates the deadenylation and clearance of several hundred maternal transcripts during zygotic stages.

MiR-430 accelerates target mRNA decay. Recent studies have suggested that miRNAs do not only inhibit productive translation but also affect target mRNA levels (*19*, *22*, *23*). To distinguish between effects on gene transcription and mRNA decay, we investigated the stability of a miR-430 reporter mRNA in the presence or the absence of miR-430. The reporter contained green fluorescent protein (GFP) and imperfect target sites for miR-430 in the 3′ untranslated region (UTR) (3xIPT-miR-430) (Fig. 1, A and B) and was injected shortly after fertilization. Reporter mRNA started to decay after 4 hours postfertilization (hpf) in wild-type embryos, following miR-430 accumulation after the onset of zygotic transcription (~2.5 hpf) (fig. S1). In contrast, reporter mRNA decay was delayed in the absence of miR-430 in

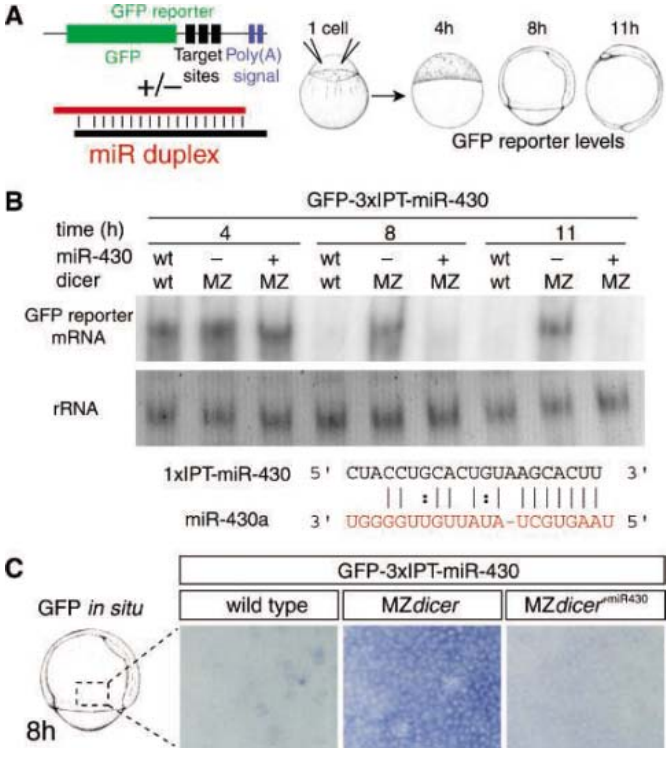

Fig. 1. MiRNAs accelerate target mRNA decay. (**A**) Experimental approach to analyze mRNA target decay in presence or absence of miRNAs. GFP reporter contains three miRNA target sites and is injected at the one-cell stage. (**B**) Northern blot of miR-430 reporter mRNA in the presence or the absence of miR-430. (**C**) In situ analysis (dark blue) of reporter mRNA in the presence or the absence of miR-430 at 8 hpf. The photos show a detail of the dorsal-lateral margin of the embryo (black rectangle, left). Note the higher reporter levels in the absence of miR-430 (fig. S2).

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A

	≥2fold	1.5-2fold	1.2-1.5fold	Unchanged	<0.8 fold	Whole Array
Probes	275	536	330	2749	713	12754
3'UTR	118	210	133	1293	359	5219*
Target sites	88	115	50	139	17	952

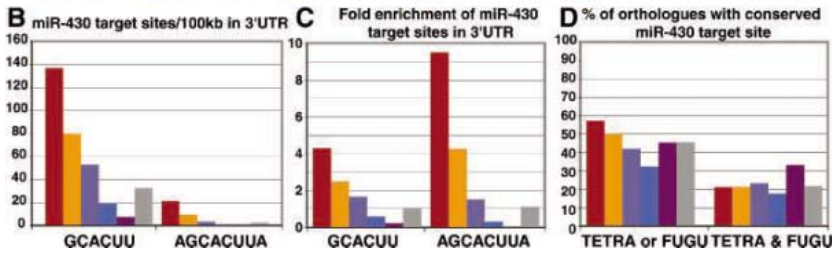

Fig. 2. MiR-430-regulated mRNAs are enriched for miR-430 target sites. (**A**) Comparison of mRNA expression levels between MZ*dicer* mutants, MZ*dicer*$^{+miR\text{-}430}$, and wild type. Columns summarize the number of probes, number of mRNAs with known 3′ UTRs, and number of mRNAs with known 3′ UTRs containing a putative miR-430 target site [defined by a hexamer (or more) sequence complementary to the miR-430 seed]. The asterisk marks the control set used for subsequent analyses. (**B** and **C**) Number (B) or -fold enrichment (C) of hexamer or octamer miR-430 target sites per 100 kb of 3′ UTR in different groups (B) and compared with the control set (C). (**D**) Percentage of mRNAs that have a hexamer (or more) miR-430 target site in the orthologous genes in *F. rubripes* (Fugu) and *T. nigroviridis* (Tetra) (fig. S3, S4, and S8).

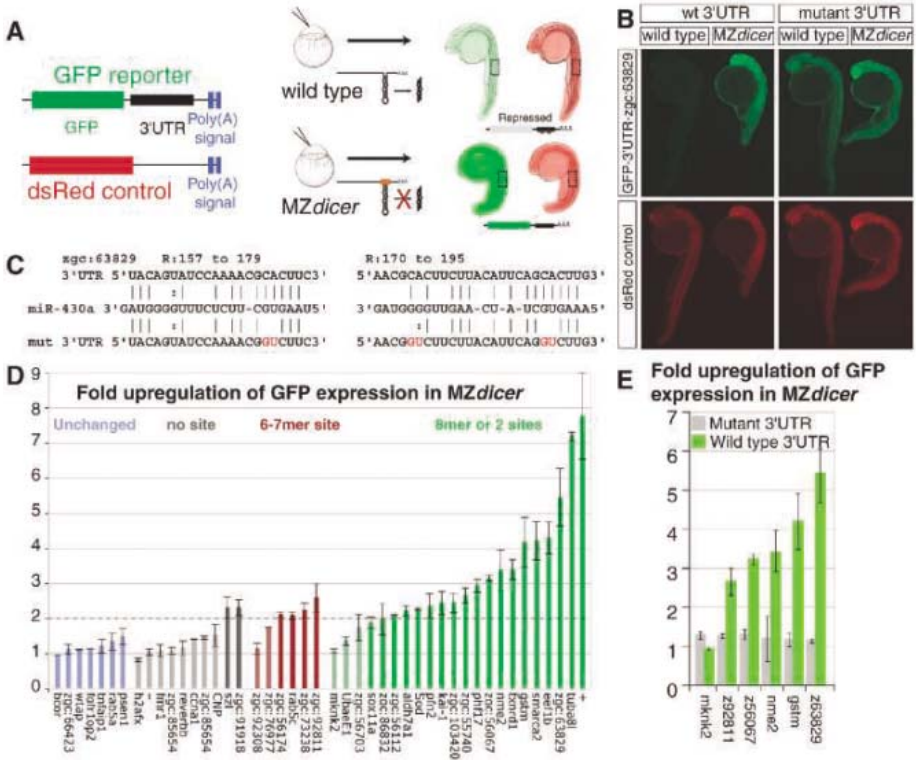

Fig. 3. miR-430 target validation. (**A**) Experimental approach. GFP reporter mRNA is co-injected with control dsRed mRNA into wild type and MZ*dicer* mutants. GFP reporter contains a 3′ UTR that is wild-type or mutant for the miR-430 target sites. (**B**) GFP reporter expression (green) and control dsRed expression (red) at 25 to 30 hpf. Wild-type reporter is repressed in wild-type embryos compared with MZ*dicer* mutants. Mutation of miR-430 target sites abolishes repression of the reporter in wild-type embryos. (**C**) Predicted pairing between wild-type or mutant target 3′ UTR and a member of the miR-430 family. (**D** and **E**) -Fold increase of GFP expression in MZ*dicer* mutants compared to wild type. Regulation was considered significant when the GFP reporter was up-regulated ≥ twofold [solid color in (D); see fig. S5, S7, and Materials and Methods for details]. Error bars indicate ± SD; $n \geq 2$; ≥15 embryos per experiment.

MZ*dicer* mutants (Fig. 1, B and C). Injection of processed miR-430 into MZ*dicer* mutants (MZ*dicer*$^{+miR\text{-}430}$) restored accelerated decay. Similar results were obtained with miR-1 and its reporter mRNA (fig. S2) (*3*). These results indicate that miRNAs can accelerate the decay of target mRNAs in vivo.

Identification of putative miR-430 targets by microarray analysis. Because miR-430 can accelerate the decay of an artificial target mRNA, we speculated that bona fide in vivo targets accumulate in the absence of miR-430. By using gene expression microarrays, we compared mRNA levels in embryos that lack miR-430 (MZ*dicer*) with those of embryos that have miR-430 (wild type and MZ*dicer*$^{+miR\text{-}430}$) (fig. S3 and table S1). More than 750 mRNAs (represented by 811 probes in the array) were present at significantly higher levels (≥1.5-fold) in MZ*dicer* mutants (Fig. 2A, fig. S3, and table S1). To determine whether these mRNAs might be direct or indirect miR-430 targets, we analyzed whether up-regulated mRNAs were enriched for putative miR-430 target sites. Such sites are complementary to the 5′ seed region of miR-430, which is crucial for target recognition (*14*, *15*, *24*–*26*). We performed this analysis for the 328 up-regulated mRNAs that had experimentally identified 3′ UTRs (Fig. 2A, fig. S3, and Materials and Methods) and a control data set containing the 5219 genes on the array with defined 3′ UTRs. On the basis of the rules developed by Lewis *et al*. (*14*), we searched for sequences complementary to a hexamer seed (positions 2 to 7 in the seed), a septamer seed, or an octamer seed (with an adenosine at the 3′ end of the target sequence) (*14*). mRNAs with increased levels in MZ*dicer* mutants had significantly more predicted target sites per 100 kb in their 3′ UTRs compared with those of the control set (Fig. 2B). This translated into a ~4- to ~10-fold enrichment compared with the control set (Fig. 2C) and a ~10- to ~30-fold enrichment when compared with the set of mRNAs that remained unchanged in the array (Fig. 2C). No enrichment was found for sequences complementary to the miRNA seeds of let-7 or miR-1 (fig. S4). These results suggest that the group of mRNAs whose levels were increased more than 1.5-fold in MZ*dicer* mutants is greatly enriched for miR-430 targets.

MiR-430 target validation. To validate putative targets, we tested miR-430-dependent regulation of reporters consisting of GFP and full-length 3′ UTRs (Fig. 3A). First, we analyzed whether GFP expression from the reporter was higher in MZ*dicer* mutants compared with that of wild type. Second, we assessed whether repression of the GFP reporter in wild-type embryos depended on the miR-430 target sites in the 3′ UTR. We co-injected GFP reporter mRNAs and control mRNAs encoding the red fluorescent protein (dsRed) and compared the GFP levels in wild type and MZ*dicer* mutants

at 25 to 30 hpf (Fig. 3, fig. S5, and Materials and Methods). We considered an mRNA to be regulated by miR-430 when the normalized GFP reporter expression in MZ*dicer* was increased more than twofold compared with wild type. Between 92% and 71% of the tested putative targets were up-regulated in vivo in the absence of miR-430 (n = 37) (Fig. 3, B to D, and figs. S5 and S6). For example, we validated 12 of 13 (92%) mRNAs with two or more target sequences complementary to miR-430 seeds, six of eight (75%) mRNAs with one octamer target site, and five of seven (71%) mRNAs with one hexamer or septamer target site. In contrast, none of the tested mRNAs that were unchanged in the array and lacked miR-430 sites were repressed in wild type compared to MZ*dicer* (n = 7).

To test for direct regulation by miR-430, we mutated two nucleotides in the predicted target site in the 3′ UTR of five putative targets (GCACUU to GGUCUU) (Fig. 3, B, C, and E, and fig. S7). This strongly reduced or abolished repression in wild-type embryos in all cases (Fig. 3E and fig. S7), suggesting that most if not all the 3′ UTRs validated in MZ*dicer* mutants are likely to be direct miR-430 targets in vivo.

Combining the microarray data, target predictions, and validation results led to the identification of a group of 101 mRNAs that have a greater than 85% probability to be direct miR-430 targets (figs. S3 and S7). Members of this group were up-regulated ≥1.5-fold in MZ*dicer* mutants and had one octamer target site or two target sites (hexa-, septa-, or octamer) in their 3′ UTRs. Even the 102 mRNAs that were up-regulated ≥1.5-fold and had one hexamer or septamer target site had a 71% probability to be miR-430 targets (figs. S3 and fig. S6). Hence, we estimate that miR-430 directly regulates ~160 mRNAs of a target set of 203 mRNAs with defined 3′ UTRs (table S1 and fig. S6). Because 3′ UTRs were only available for roughly half (328 of ~750) of the up-regulated mRNAs, we extrapolate that miR-430 might directly regulate more than 300 of these mRNAs (fig. S6). If we also take into account that the array covers less than half of all predicted zebrafish genes, we estimate that miR-430 directly regulates several hundred target mRNAs during early zebrafish development.

MiR-430 targets are not preferentially conserved. Computational identification of miRNA targets often relies on the principle that conserved miRNAs have conserved targets (*1*, *7*). We therefore asked how many of the 203 genes in the zebrafish miR-430 target set have a hexamer miR-430 target site in the 3′ UTR of orthologous genes in two other teleosts, *Fugu rubripes* and *Tetraodon nigroviridis* (fig. S8 and table S1). As described in Materials and Methods, we estimate that ~50% of our experimentally identified target sites are found in the orthologous genes in either *Fugu* or *Tetraodon* and only ~25% are conserved in all three species (Fig. 2D and fig. S8). Regulation mediated by the 3′ UTR of conserved and nonconserved targets resulted in similar posttranscriptional regulation of the GFP reporter (fig. S8). These results suggest a very rapid evolution of the miR-430 target set and indicate that a large fraction of bona fide targets for miR-430 and other miRNAs (*18*) would be missed if conservation were used as the sole criterion.

Most miR-430 target mRNAs are expressed maternally. To investigate whether the 203 mRNAs in the miR-430 target set share specific features, we analyzed their expression profile and gene ontology (GO). No significant differences in the distribution of GO classes were found for genes in the target set compared to the entire genome (fig. S9). We then compared the expression profile of genes in the miR-430 target set with genes in the control set that were expressed during early embryogenesis. About 40% of the target set mRNAs but only ~10% of the control set mRNAs were present at high levels at 1.5 hpf but at low levels at 5 and 9 hpf (Fig. 4A and fig. S10). These are mRNAs deposited into the egg by the mother before fertilization, because the genome is transcriptionally silent until ~2.5 hpf (*21*, *27*). In the absence of miR-430, the maternal transcripts in the target set accumulated to higher levels than in wild-type embryos (table S1). In addition to the fourfold enrichment of predominantly maternal transcripts in the miR-430 target set, ~40% of predominantly maternal mRNAs (162 out of 402) were predicted to have miR-430 target sites compared with only 18% in the control set (Fig. 4C and fig. S10). Hence, not only was the miR-430 target set enriched for maternal mRNAs, but maternal mRNAs were also enriched for miR-430 target sites. Another ~50% of the target mRNAs were expressed both maternally and zygotically. In particular, half of these targets maintained steady levels zygotically (Fig. 4B and fig. S10) but accumulated to higher levels in the absence of miR-430 (table S1). Taken together, the analysis of miR-430 targets indicates that miR-430 accelerates the clearance of several hundred maternal and maternal-zygotic mRNAs.

MiR-430 promotes target mRNA deadenylation. The polyadenosine [poly(A)] tail stabilizes mRNAs and enhances cap-dependent translation (*28*–*31*). Upon fertilization, many maternally deposited mRNAs become polyadenylated and competent for translation, whereas deadenylation triggers translational silencing and decay (*28*–*30*). We therefore tested whether miRNA-induced target decay correlates with changes in the length of the poly(A) tail of reporter and endogenous target mRNAs. Time course analysis of injected target mRNAs revealed that polyadenylation is followed by rapid deadenylation that is induced by miR-430 and results in complete deadenylation at about 6 hpf (~3 hours after the onset of miR-430 accumulation) (Fig. 5 and fig. S11). Three controls indicated that rapid deadenylation is miRNA-mediated. First, mutation of miR-430 target sites in the 3′ UTR delayed target deadenylation (Fig. 5, A to D). Second, deadenylation of target mRNAs is delayed in MZ*dicer* mutants, similar to non-miRNA targets or GFP alone (Fig. 5, D to F,

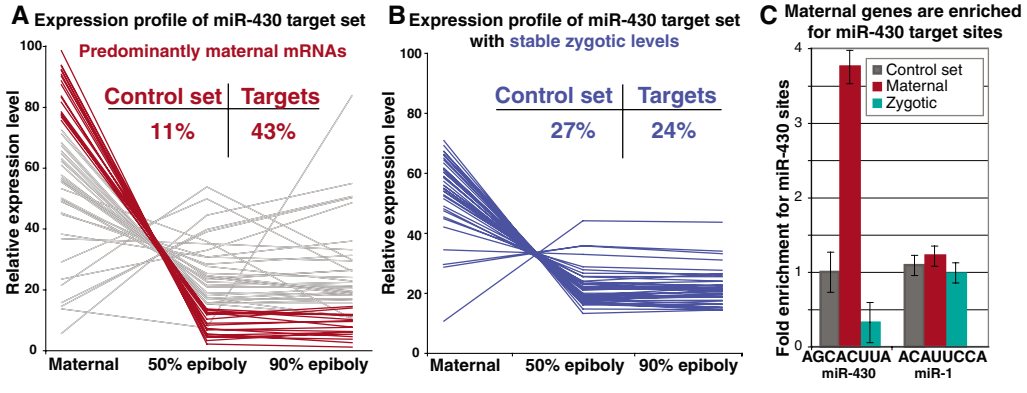

Fig. 4. MiR-430 facilitates the clearance of maternal transcripts. (**A** and **B**) Relative expression profile of mRNAs at 1.5 (maternally provided mRNAs), 5, and 9 hpf. (**A**) Expression profile of 50 mRNAs in the miR-430 target set. Predominantly maternal mRNAs are highlighted in red. The miR-430 target set is fourfold enriched for predominantly maternal mRNAs when compared with a random set of mRNAs (fig. S10). (**B**) Expression profile of 50 mRNAs in the miR-430 target set that are present at different levels maternally but show stable levels during zygotic stages (blue). The mRNAs in the miR-430 target set accumulate to higher levels in the absence of miR-430 (table S1). (**C**) -Fold enrichment of octamer target sites for miR-430 and miR-1 in the control set, predominantly maternal mRNAs, and predominantly zygotic mRNAs. About 40% of the predominantly maternal mRNAs have a hexamer miR-430 target site (see Materials and Methods for details regarding maternal and zygotic mRNAs).

and fig. S11). Third, injection of miR-430 into MZ*dicer* mutants accelerates target deadenylation (Fig. 5, D and F). Similar results were observed for different endogenous and reporter miR-430 targets (fig. S11) as well as reporters partially complementary to miR-1 (Fig. 5G).

To test whether miRNA-induced target deadenylation is a secondary effect of nonproductive translation, we repressed GFP reporter expression by using morpholino antisense oligonucleotides that hybridize to the translational start site. This did not cause reporter mRNA decay or deadenylation to the same extent as the miRNA did (Fig. 5G). Furthermore, miRNA-induced deadenylation still occurred in the absence of translation (Fig. 5G). Taken together, these results indicate that miR-430 accelerates target deadenylation and mRNA decay.

The study of miR-430 in vivo targets provides three major insights. First, miR-430 directly regulates several hundred target mRNAs during early embryogenesis. These targets are up-regulated in MZ*dicer* mutants, contain miR-430 target sites, and can be validated in vivo. Although the number of identified miR-430 targets is high, it is likely that there are additional targets that were not identified in our approach. These targets might include mRNAs that are expressed at low levels, regulated by miR-430 during other developmental stages, or regulated at the level of translation rather than transcript stability. Our results provide in vivo support for in silico and cell culture studies that have predicted large sets of putative miRNA targets (*7*, *11–19*, *31*). However, 50% of the miR-430 target set would have been missed by relying on conservation, a criterion that is often used in computational approaches. Moreover, it remains unclear how many of the in silico or cell culture targets are biologically relevant targets in vivo. In contrast, in vivo approaches using miRNA loss-of-function combined with target validation identify bona fide targets.

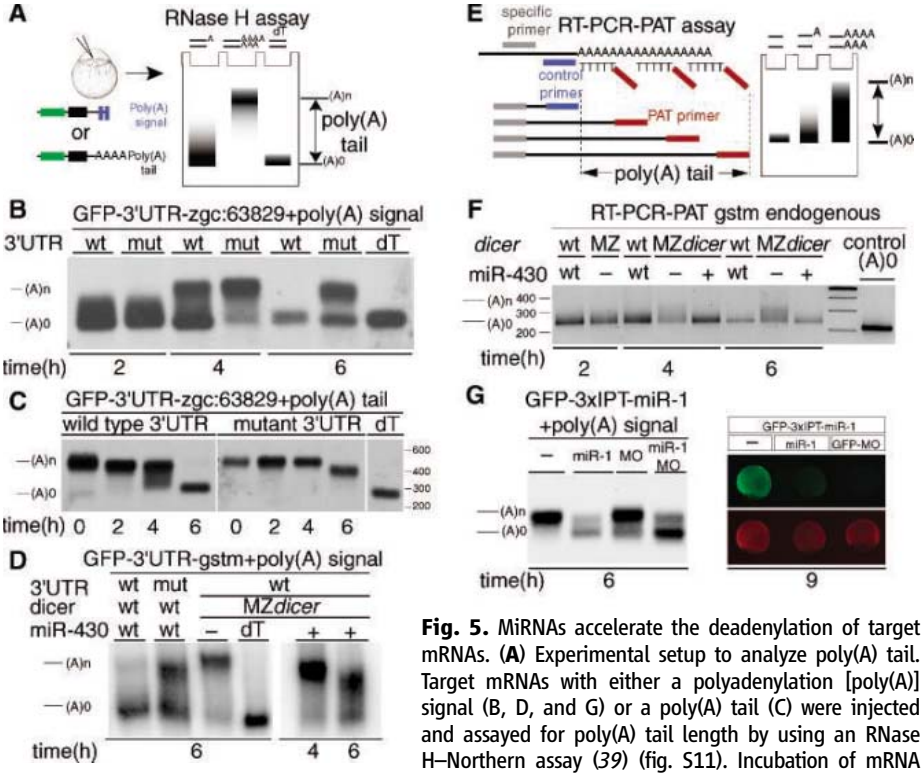

Fig. 5. MiRNAs accelerate the deadenylation of target mRNAs. (**A**) Experimental setup to analyze poly(A) tail. Target mRNAs with either a polyadenylation [poly(A)] signal (B, D, and G) or a poly(A) tail (C) were injected and assayed for poly(A) tail length by using an RNase H–Northern assay (*39*) (fig. S11). Incubation of mRNA with oligodeoxythymidine (dT) and RNase H tests the mobility of the deadenylated RNA fragment. (**B** to **D**) Northern analysis of poly(A) tail length of GFP reporter mRNA with wild-type (wt) or mutant (mut) target sites in the 3′ UTR in the presence or absence of miR-430. GFP reporter mRNA is polyadenylated between 0 and 4 hpf (B). Deadenylation of wild-type GFP reporter is accelerated compared with that of GFP reporters with mutated miR-430 target sites (B and C) and in wild type compared with MZ*dicer* mutants (D). Accelerated deadenylation can be partially rescued in MZ*dicer* mutants by injecting miR-430 duplexes (D) (fig. S11). (**E**) Method used to detect poly(A) tail length of endogenously expressed target mRNAs, reverse transcription polymerase chain reaction (RT-PCR) poly(A) test (PAT). The length of the smear reveals the approximate size of the poly(A) tail. (**F**) Time course RT-PCR PAT of endogenous miR-430 targets in the presence of endogenous (wt), injected miR-430 (+), or absence of miR-430 (MZ*dicer*, –). Note the shortening of the poly(A) tail in the presence of miR-430 (see Materials and Methods for details). (**G**) RNaseH-Northern assay of a GFP reporter for miR-1 in the presence or absence of translational repression by antisense morpholino (MO). MiR-1 and MO repress GFP translation (green), but miR-1 preferentially accelerates deadenylation.

Second, our results suggest a critical role for miR-430 in the maternal-to-zygotic transition during embryogenesis. In all animals, the mother deposits mRNAs into the egg. Upon fertilization, maternal mRNAs are activated and translated, whereas the genome is transcriptionally silent until a later stage (*21*, *28–30*). Because maternally provided transcripts cannot be repressed at the transcriptional level, posttranscriptional and posttranslational mechanisms are required to regulate their activity (*28–30*). MiR-430 is expressed at the onset of zygotic transcription and accelerates the deadenylation and decay of a large set of maternal mRNAs (fig. S12). The accumulation of these maternal transcripts is the likely cause of the developmental delay and gastrulation defects observed in MZ*dicer* mutants (*3*). MiRNA-induced clearance of maternal mRNAs might be a universal mechanism to regulate the maternal-to-zygotic transition. Our analysis of miR-430 and its targets might also have wider implications for miRNA function during development. MiR-430 is involved in a developmental transition (maternal-to-zygotic), but, even without miR-430 and despite the delayed clearing of maternal mRNAs, the embryo can still activate its zygotic program (e.g., embryonic patterning). Hence, lack of miR-430 does not arrest the embryo in a maternal state but results in a mixed maternal-zygotic state. This suggests that miR-430 and other miRNAs might not exclusively function as switches from one state (B) to the next state (C) but might sharpen developmental transitions and counteract the formation of mixed states (B-C) (fig. S12). This model has important implications for the role of miRNAs in disease. Premature expression of miRNAs might aberrantly target mRNAs required to specify state B, thus maintaining cells in an earlier state A (fig. S12). Such a mechanism might account for the miRNA-mediated induction of cancer (*2*). Our findings also provide a framework to interpret recent studies that have indicated that specific miRNAs and their predicted target mRNAs tend to be expressed in a mutually exclusive manner (*17*, *18*). These and previous results (*19*, *32*) have led to the hypotheses that miRNAs confer robustness by targeting erroneously transcribed genes or by preventing premature or prolonged mRNA accumulation. Our results provide in vivo support for the latter hypothesis, suggesting that miRNAs target mRNAs that might otherwise persist during later stages. In addition, a fraction of the target mRNAs continues to be coexpressed with miR-430 after the maternal-to-zygotic transition. These target mRNAs are not eliminated but modulated by miR-430. Thus, miR-430 accelerates the clearance of maternal mRNAs and dampens the levels of maternal-zygotic mRNAs.

Third, our study suggests that miR-430 accelerates deadenylation of target mRNAs and thus provides a potential mechanism for miRNA function. The poly(A) tail confers mRNA stabil-

ity and stimulates translation via the interaction of poly(A) binding protein (PABP) with the 5′ m7G cap (*28*–*30*). Previous studies have found that miRNAs inhibit Cap-dependent translation (*33*) and induce mRNA degradation (*19*, *22*, *23*, *34*). We therefore postulate that miRNA-induced deadenylation is one of the mechanisms by which miRNAs enhance target decay and repress productive translation (fig. S12). Although it remains to be determined whether deadenylation is the primary step in mRNA regulation, we observed that block of translation does not accelerate mRNA deadenylation to the same extent as the miRNA does. It is therefore conceivable that miRNAs induce the deadenylation of their targets, which results in the block of translation and the recruitment to processing bodies (P-bodies), where mRNAs are decapped and degraded (*33*–*38*). Taken together, our results suggest that miRNAs promote the deadenylation and decay of hundreds of target mRNAs and thus sharpen and accelerate the transition between developmental states.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1122689/DC1
Materials and Methods
Figs. S1 to S12
Table S1(≥1.5fold).xls
Targets-tested.doc

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Evolution of the Eastern Tropical Pacific Through Plio-Pleistocene Glaciation

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A tropical Pacific climate state resembling that of a permanent El Niño is hypothesized to have ended as a result of a reorganization of the ocean heat budget ~3 million years ago, a time when large ice sheets appeared in the high latitudes of the Northern Hemisphere. We report a high-resolution alkenone reconstruction of conditions in the heart of the eastern equatorial Pacific (EEP) cold tongue that reflects the combined influences of changes in the equatorial thermocline, the properties of the thermocline's source waters, atmospheric greenhouse gas content, and orbital variations on sea surface temperature (SST) and biological productivity over the past 5 million years. Our data indicate that the intensification of Northern Hemisphere glaciation ~3 million years ago did not interrupt an almost monotonic cooling of the EEP during the Plio-Pleistocene. SST and productivity in the eastern tropical Pacific varied in phase with global ice volume changes at a dominant 41,000-year (obliquity) frequency throughout this time. Changes in the Southern Hemisphere most likely modulated most of the changes observed.

A notable inflection point in the global benthic oxygen isotope ($\delta^{18}O$) record at ~3 million years ago (Ma) marks the beginning of the Plio-Pleistocene transition (*1*). During the preceding early Pliocene interval (3 to 5.3 Ma), there was little or no ice in the Northern Hemisphere (*2*), and global mean surface temperatures were ~3°C warmer than they are today (*3*). However, at ~3 Ma, benthic $\delta^{18}O$ values and ice-rafted debris in the North Atlantic and North Pacific signal two substantial changes in high-latitude climate (*4*–*6*). The Northern Hemisphere began a period of long-term growth in continental ice, most rapidly between about 3 and 2 Ma. At the same time, the variability of high-latitude climate increased markedly, as seen by the growing amplitude of 41,000-year (41-ky) obliquity cycles in benthic $\delta^{18}O$ beginning ~3 Ma (*7*). What was the manifestation of this climatic transition in the tropics? One emerging theory is that the tropical ocean shifted from a state much like permanent El Niño before ~3 Ma to its modern, more La Niña–like state after ~3 Ma (*8*–*11*).

In the modern ocean, the ventilated thermocline (the strong vertical thermal gradient between warm surface waters and cool deep waters) in the EEP brings cold, nutrient-rich waters to the surface, which are initially derived from the sinking of mid- to high-latitude surface waters in the Southern and Northern hemispheres (*9*, *12*–*14*). This outcropping of cold, nutrient-rich water gives rise to high productivity in the EEP and sets up east-west SST and atmospheric pressure gradients, which reinforce and are reinforced by the Trade Winds. At present, mean annual SSTs are 23°C in the EEP and 29°C in the western equatorial Pacific (WEP), yielding a modern surface temperature difference of 6°C (*15*). The resulting temperature gradient and associated pressure gradients drive the strong east-west atmospheric circulation pattern (Walker Circulation). A disruption of Walker Circulation gives rise to El Niño conditions, in which weaker easterly Trade Winds and a deeper thermocline in the EEP result in a warming of surface waters and a concomitant decline in biological productivity.

A recent theory (*9*) connects the posited transition from a permanent El Niño to a La Niña climate state to a fundamental change in linkages between the high- and low-latitude ocean on long-term time scales (>10^6 years) and orbital time scales (10^4 to 10^5 years). According to this

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theory, the gradual cooling of the deep ocean culminated ~3 Ma in a shoaling of the ventilated thermocline and gave rise for the first time to cold tropical upwelling zones, ending the permanent El Niño. In this new La Niña–like state, low- and high-latitude climates became linked because heat gained in low-latitude upwelling zones must be balanced by heat lost to the atmosphere at high latitudes. According to the Philander and Fedorov (*9*) theory, one manifestation of the end of the permanent El Niño configuration should be the appearance of 41-ky obliquity cycles in tropical upwelling zones. High obliquity would result in deglaciated high latitudes and El Niño–like conditions in the tropical Pacific (i.e., a deeper EEP thermocline), which should correspond to warmer and less productive conditions in the EEP. In contrast, low obliquity would induce glaciation and La Niña–like conditions (i.e., a shallower EEP thermocline), which should correspond to observations of a colder, more productive EEP.

Here, we test the hypotheses that the EEP upwelling zone and the low-latitude obliquity response arose synchronously during the late Pliocene (~3 Ma). Using the alkenone unsaturation index ($U^{k'}_{37}$)—a faithful recorder of mean annual SST (*16*, *17*) and changes in alkenone concentration (C_{37} Total), which is a proxy for phytoplankton productivity (*18*, *19*)—we monitored the evolution of two defining characteristics of the modern EEP: its cool SST and high biological productivity. Our data set from Ocean Drilling Program (ODP) Site 846 (3°S, 91°W; water depth 3296 m) is 5 million years (My) long and provides the longest high-resolution (~3-ky) record of Earth surface temperatures extant. This record allows us to evaluate linkages between different aspects of the Earth's climate system on both orbital time scales (10^4 to 10^5 years) and supra-orbital time scales (>10^6 years). Our results show that obliquity has modulated EEP surface conditions for at least the past 5 My. However, the EEP obliquity response increased substantially starting ~3 Ma as the EEP upwelling zone cooled and the SST gradient between the eastern and western Pacific grew.

EEP surface ocean variations. The Site 846 $U^{k'}_{37}$ SST record documents a long-term cooling of ~1°C/My with temperatures as high as 28°C in the early Pliocene and as low as 20°C in the late Pleistocene (Fig. 1B). Average SST at Site 846 (26°C) during the early Pliocene (3 to 5 Ma) approached values typical of the region during modern El Niño events (~27°C). Much of the Site 846 SST record is notably similar in trend and structure to the Site 846 benthic $\delta^{18}O$ record (*20*, *21*) (Fig. 1), illustrating that substantial Plio-Pleistocene cooling extended into the tropics. Similar to the benthic $\delta^{18}O$ record, SST variations increased in amplitude ~3 Ma (Fig. 1, B and C). Low-amplitude glacial-interglacial temperature cycles (average amplitude ~1°C) dominate the early Pliocene (3 to 5 Ma) SST record. Amplitudes increased during the late Pliocene with variations as large as 5°C (Fig. 1B). In contrast to the benthic $\delta^{18}O$, in which the major inflection point occurs at ~3 Ma and corresponds to substantial ice growth or high-latitude cooling, the $U^{k'}_{37}$ SST record shows that important changes happened before ~3 Ma (Fig. 1, B and C). Cooling in the surface ocean of the EEP started at least 1 My before the intensification of Northern Hemisphere glaciation (NHG), implying that while the growth of Northern Hemisphere ice sheets undoubtedly played a major role as a climatic feedback during the Plio-Pleistocene transition, it did not force or initiate EEP cooling (Fig. 1, B and C).

Our results from the EEP confirm the results of Wara *et al*. (*11*) (who found much warmer, more El Niño–like conditions, in the EEP based on Mg/Ca paleothermometry) and refute the conclusion of Rickaby and Halloran (*22*) (who found that conditions in the tropical Pacific during the Pliocene were cool and La Niña–like). However, our orbitally resolved SST time series shows that Site 846 SST estimates are an average of ~2°C cooler than those of Wara *et al*. (*11*) at Site 847 and suggests that EEP cooling began not at ~2.5 Ma, as suggested by Wara *et al*. (*11*), but at least as early as 4.3 Ma (Fig. 1B). These discrepancies may stem from actual differences in the dynamics between study sites several hundred kilometers apart in the EEP, from changes and differences in the ecology of the species responsible for the paleoclimate indexes measured (haptophyte algae for the $U^{k'}_{37}$ index versus the planktonic foraminifer *Globigerinoides sacculifer* for the Mg/Ca proxy) (*23*), from differences between the calibrations for alkenone and Mg/Ca paleothermometry, or from aliasing of orbital-scale variability in the Wara *et al*. (*11*) record, which has lower resolution.

Overall, high alkenone fluxes in sediments from ODP Site 846 [average mass accumulation rates (MAR) of 61 μg/cm²/ky] in comparison to alkenone MARs reported from other oceanic locations (0.1 to 35 μg/cm²/ky) (*18*, *24*) suggest that the EEP has been a locus of high biological productivity for at least the past 5 My. Alkenone concentration variations highlight a regime between 2.9 and 1.6 Ma of distinctly higher productivity than came before or after [Fig. 1A, supporting online material (SOM) text, and fig. S1]. Alkenone concentrations during this interval were three times and two times as high as average early Pliocene and Pleistocene values, respectively. This finding is corroborated by a similar maximum in the MAR of biogenic carbonate in the subtropical eastern Pacific between 2.9 and 1.7 Ma (*8*). On glacial-interglacial time scales, higher alkenone concentrations almost universally occur during cold intervals (Fig. 1, A and B), indicating that temperature and productivity were tightly coupled on orbital time scales (≤10^5 years). Increased variance in both the SST and $\delta^{18}O$ records (Fig. 2, A and B) accompanies the marked increase in productivity at ~2.9 Ma (Fig. 1A). The de-

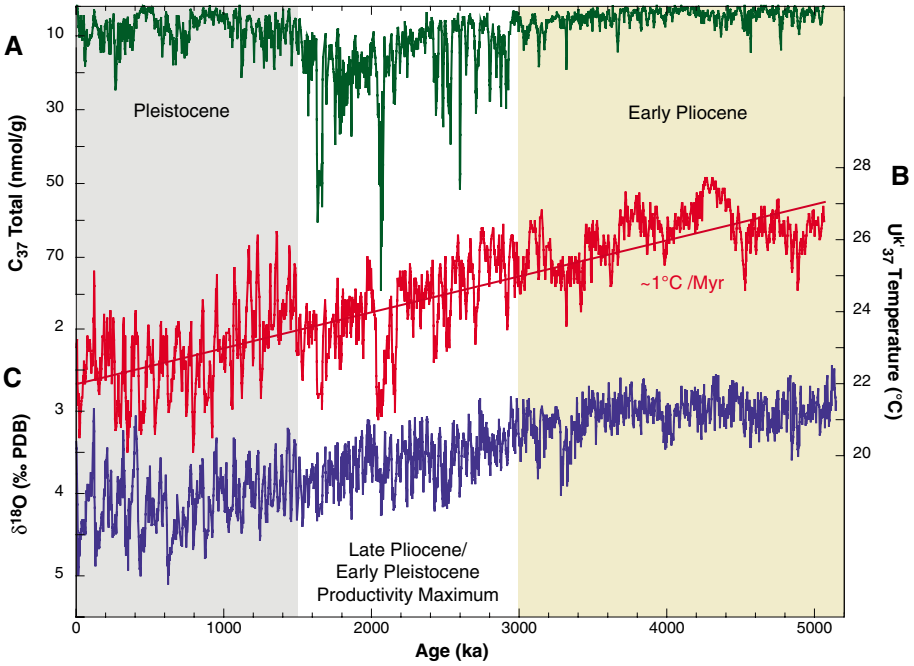

Fig. 1. Paleoclimatic proxies from ODP Site 846. (**A**) Concentration of C_{37} alkenones (nmol/g) (green line) (note the inverted axis), (**B**) $U^{k'}_{37}$ sea surface temperature (°C) (red line), and (**C**) benthic $\delta^{18}O$ in parts per thousand PD belemnite (PDB) (note the inverted axis) (*20*, *21*). All data are correlated to the LR04 Stack (*7*) with the use of the Match 2.0 program (*32*). The early Pliocene is shaded yellow, a white background defines the interval of maximum EEP productivity, and the portion of the Pleistocene after the productivity maximum is shaded gray. ka, thousands of years ago.

cline in productivity at ~1.6 Ma corresponds to a slight warming of SST (~1°C) during the interval from 1.6 to 1 Ma (Fig. 1, A and B).

Evolutionary spectra of Site 846 $\delta^{18}O$, $U^{k'}_{37}$ temperature, and C_{37} Total show that obliquity power plays a dominant role in all three records over the past 5 My (Fig. 2, A to C). The 1.2-My modulation (i.e., the envelope of the obliquity signal), notable in the orbital forcing as a series of alternating intervals of strong versus weak spectral density (Fig. 2D), is discernable in all three climatic responses (Fig. 2, A to C). Similar to $\delta^{18}O$, the $U^{k'}_{37}$ SST record shows a pronounced increase in obliquity band variance toward the present, starting at ~3 Ma (Fig. 2, A and B). In contrast, the C_{37} Total productivity record responds more faithfully to obliquity pacing, with a strong response at 41 ky observed even before the intensification of NHG (~3 Ma) (Fig. 2C).

In the obliquity band, all three proxies are coherent for the majority of the past 5 My (Fig. 3, A to C). The exceptions generally coincide with obliquity nodes (intervals of low obliquity amplitude) when obliquity band climatic forcing is weak (Fig. 3D). SST and C_{37} Total are anticorrelated (cold temperatures correspond to high productivity) throughout the entire interval, as shown by the nearly constant phase relationship –1 ± 1.5 ky (±2σ) between SST and C_{37} Total (Fig. 3A). After ~2.5 Ma, both temperature and C_{37} Total slightly lead (3 ± 1.5 ky and 4 ± 1.75 ky, respectively) benthic $\delta^{18}O$ in the obliquity band (Fig. 3, B and C, and SOM text). Before this time, all proxies are more nearly in phase (Fig. 3, B and C). The slight increase in lag of benthic $\delta^{18}O$ relative to changes in sea surface indices after 2.5 Ma may testify to the greater thermal inertia of Northern Hemisphere ice sheets as they rapidly expanded during the intensification of NHG (*25*).

Mechanisms for EEP sea surface change. What mechanisms gave rise to these orbital-scale and longer term climatic variations in the EEP sea surface? Plausible mechanisms for altering EEP SST are changing (i) local insolation, (ii) the east-west tilt of the thermocline, (iii) the average depth of the thermocline, or (iv) the composition of the atmosphere (i.e., changing greenhouse gas concentrations). EEP productivity could be changed by altering (i) the east-west tilt of the thermocline, (ii) the average depth of the thermocline, or (iii) the nutrient content of the source waters. Some of these mechanisms result in coupled changes in SST and productivity. Others may lead to independent changes in one proxy record without a corresponding change in the other. For example, changing the depth or tilt of the thermocline should result in changes in both SST and productivity, whereas changes in atmospheric composition can affect ocean surface temperature without modifying biological productivity. However, these mechanisms are not necessarily mutually exclusive. The ODP Site 846 record suggests that temperature and productivity of the eastern Pacific upwelling zone were tightly coupled on orbital time scales, but only weakly coupled on long time scales, implying that different mechanisms may drive changes in EEP surface conditions on these two time scales.

Orbital time scales. At low latitudes, changes in seasonal insolation caused by variations in precession [~10 watts per m^2 (Wm^{-2})] are far greater than those driven by obliquity oscillations (<1 Wm^{-2}), suggesting that important low-latitude climatic processes (such as Trade Winds and monsoons) should vary at precessional periods. However, our low-latitude sea surface records have only weak precessional power (Fig. 2, B and C), implying a marginal role for low-latitude processes in orbital-scale variations in EEP climate over the past 5 My. Instead, obliquity oscillations dominated EEP records of both productivity and temperature for most of this time and were nearly in phase (average lead of 2 to 3 ky) with changes in benthic $\delta^{18}O$ wherever the signals were coherent (Fig. 3, B and C). Although we cannot precisely measure the phase relationship of geological responses to orbital obliquity forcing, the unambiguous coupling of a colder and more productive EEP with enriched benthic $\delta^{18}O$ indirectly demonstrates a consistent link between low orbital obliquity, which favors ice sheet growth in high latitudes, and the cold EEP we document here (SOM text). As previously documented for the early Pleistocene (*26*), SST variations were out of phase with low-latitude obliquity insolation changes throughout the entire time series (i.e., the EEP cooled in a low orbital obliquity configuration, which actually provided more solar heating to the tropics).

The tight coupling between SST and productivity implies that high-latitude obliquity variations adjusted either the tilt or depth of the EEP thermocline. These relationships are consistent with the obliquity-related changes in thermocline depth predicted by Philander and Fedorov (*9*). When obliquity was high, EEP conditions were El Niño–like, with warm SSTs and low productivity, implying a deep mean depth of the thermocline; when obliquity was low, EEP conditions were La Niña–like, with cold SSTs and high productivity, implying a shallow mean depth of the thermocline.

The El Niño model might successfully account for SST and productivity changes observed

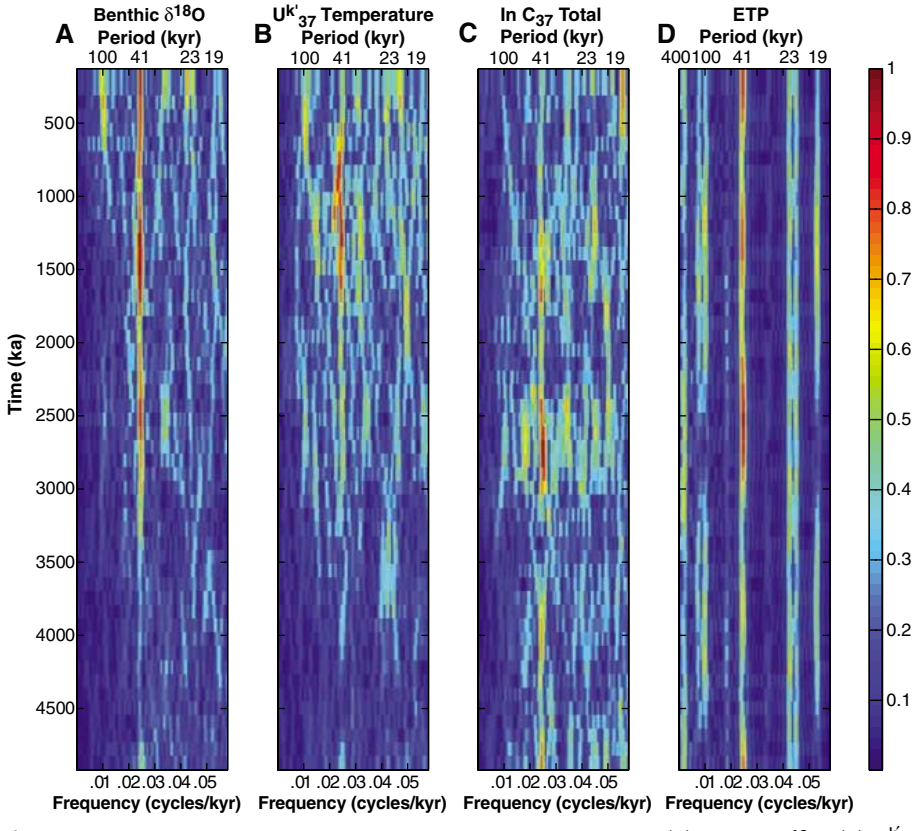

Fig. 2. Evolutionary spectra of ODP 846 paleoclimate proxies and ETP. (**A**) Benthic $\delta^{18}O$, (**B**) $U^{k'}_{37}$ temperature, and (**C**) natural logarithm (ln) C_{37} Total (alkenone concentration) (*18*, *19*). (**D**) Eccentricity, obliquity (tilt), and precession (ETP) (*33*). ETP is an illustration of changes in orbital variations through time. These spectra were computed with the use of a fast Fourier transform (FFT) with a 600-ky window and 85% overlap between windows. All responses were interpolated to even intervals of 1-ky resolution and prewhitened (except ETP) before being subjected to the FFT. The same major spectral features are reproducible with the use of the Blackman-Tukey method, indicating that the results produced in this analysis are not methodology dependent.

in the EEP considered in isolation, but recent SST estimates from the Pleistocene western Pacific suggest that the El Niño model incompletely describes the larger tropical Pacific on orbital time scales. Two recent studies have shown that during the early Pleistocene glacial-interglacial SST variations in the western equatorial Pacific were dominated by 41-ky cycles, with warmings and coolings of similar amplitude and phasing as those in the East (*27*, *28*) (fig. S2). In the modern ocean, El Niño results in a SST seesaw in the equatorial Pacific Ocean. One would therefore expect to find opposing SST responses between east and west in a pure El Niño model of 41-ky cyclicity. Instead, the similarity in glacial-interglacial phasing and amplitude of SSTs between the EEP and the WEP during the Pleistocene (fig. S2) implies the existence of a mechanism capable of synchronizing tropical SST changes in a manner not consistent with a simple vertical movement or rocking of the thermocline. We concur with Medina-Elizalde and Lea (*27*), who concluded that changing concentrations of atmospheric greenhouse gases were an important component of orbital-scale SST changes during the Pleistocene. We hypothesize that in addition to movements of the thermocline, greenhouse forcing likely played a major role in driving orbital-scale SST variability in the EEP throughout the past 5 My.

Long time scales. Despite the occurrence of major global climatic changes at ~3 Ma, which in the EEP were recorded as a marked increase in obliquity band variance of both $\delta^{18}O$ and EEP SST (Fig. 2, A and B) and a threefold increase in alkenone concentration (Fig. 1A), the obliquity band coherency and phase relationships between EEP SST, productivity, and $\delta^{18}O$ remained essentially unchanged (Fig. 3, A to C). If we accept benthic $\delta^{18}O$ as a high-latitude proxy, reflecting a combination of ice volume and deep-ocean temperature changes that are imparted at high latitudes, then the long-term constancy of these relationships suggests that the "plumbing" of the EEP upwelling system has not changed appreciably over the past 5 My and indicates that a strong link in the obliquity band existed between high- and low-latitude climates well before 3 Ma. The persistence of obliquity responses suggests that, consistent with the theory, but in contrast to the timing proposed by Philander and Fedorov (*9*), the high latitudes continued to modulate EEP surface conditions so long as a west-east SST gradient persisted (i.e., for at least the past 5 My) (fig. S3). Our results do not invalidate the fundamental tenet of the Philander and Fedorov (*9*) theory, which states that a major reorganization of the sources and sinks of the oceanic heat budget occurred in association with long-term cooling of the deep ocean, but they suggest that the onset of such a reorganization must be moved to before 5 Ma.

Given that Southern Hemisphere cooling and glaciation preceded that of the Northern Hemisphere by millions of years (*1*), we suggest that the critical establishment of a high- and low-latitude link by means of the thermocline may have depended more on oceanographic changes in the Southern, rather than Northern, Hemisphere. One of the most prominent observations implicating the role of the Southern Ocean in the evolution of EEP surface conditions is the poor correspondence of the high productivity interval between about 2.9 and 1.6 Ma (Fig. 1C) to either the local SST, as inferred from alkenone paleotemperatures, or to east-west equatorial Pacific gradients, as inferred from comparing our SST estimates at Site 846 with the (more sparsely sampled) Mg/Ca paleotemperature estimates from the Western Pacific Warm Pool (ODP Site 806) (*11*) (fig. S3). Substantial changes in EEP productivity occur in the modern El Niño Southern Oscillation cycle in response to shoaling or deepening of the equatorial thermocline and strengthening or weakening of the Trade Winds. An El Niño model would therefore predict that higher past productivity should coincide with colder EEP SST and enhanced east-west gradients. At the orbital scale, we do indeed observe the association of colder SST and higher past productivity. Yet, EEP productivity increased abruptly at 2.9 Ma without a similar anomaly of cold SST at Site 846, and the interval in which the west-east SST gradient became the strongest (past 1.5 My) (*8*, *11*) (fig. S3), counter to expectations, corresponds to a substantial decline in EEP surface productivity (Fig. 1A). Both of

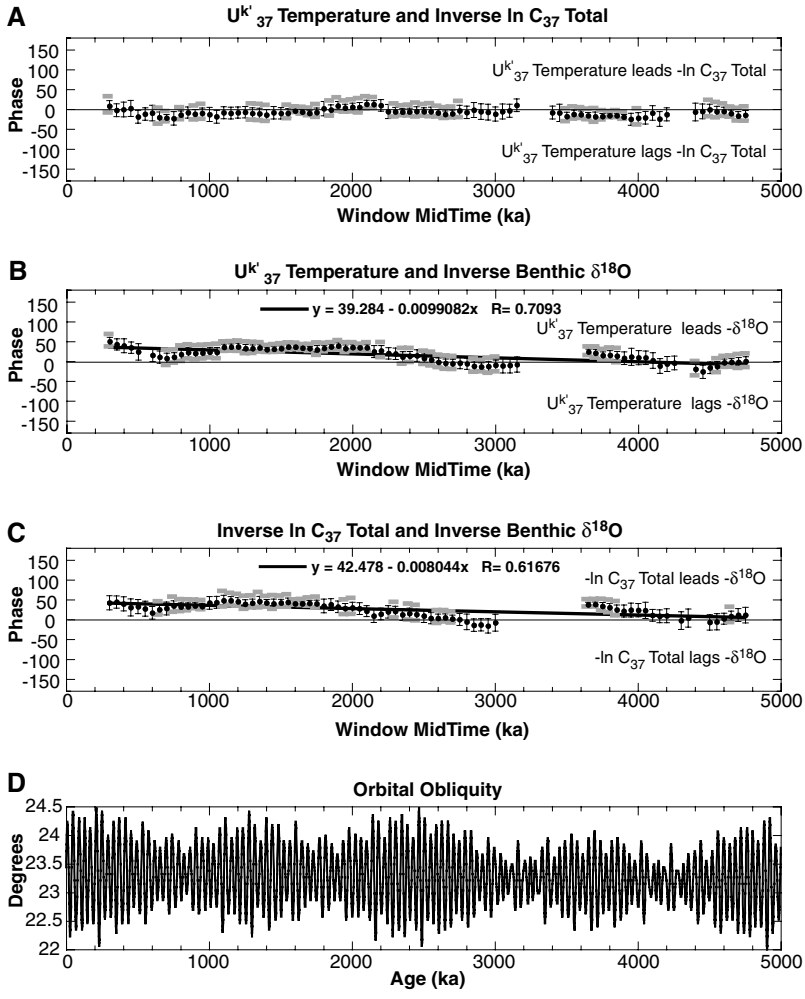

Fig. 3. Obliquity band (41-ky) phase and coherency relationships and orbital obliquity. (**A**) $U^{k'}_{37}$ temperature (SST) and alkenone concentration (productivity) (–ln C_{37} Total), (**B**) $U^{k'}_{37}$ temperature (SST) and benthic $-\delta^{18}O$ (ice volume), (**C**) alkenone concentration (productivity) (–ln C_{37} Total) and benthic $-\delta^{18}O$ (ice volume), and (**D**) orbital obliquity (*34*). Intervals that are coherent at the 80% confidence level are shown with thin black error bars and those that are coherent at the 95% confidence level are shown with thick gray bars. Because the alkenone concentrations have a log-normal rather than Gaussian distribution, we used the natural logarithm of alkenone concentration (C_{37} Total). We use the inverse of both benthic $\delta^{18}O$ and alkenone concentration in our coherency and phase analyses to be consistent with paleoclimatic convention and the axes in Fig. 1. Before coherency and phase analysis, all records were interpolated to even intervals of 1-ky resolution. Phases were computed with the use of the Arand Program iterative spectra feature with a 600-ky window and 300 lags. Regression lines [thick black lines in (B) and (C)] show the trend toward increasing phase difference toward the present.

these observations are inconsistent with a change in the tilt or depth of the thermocline uniquely determining the extent of EEP productivity.

We hypothesize that nutrient availability, a characteristic of the source waters, rather than a change in wind-driven upwelling strength, controlled productivity variations in the EEP on long time scales. Today, the cold, nutrient-rich waters of the ventilated thermocline that are upwelled in the EEP are sourced by intermediate and mode waters from the high latitudes of the North Pacific and the Southern Ocean (*12*, *14*), with greater contributions from the Southern Hemisphere (*13*). A crash in productivity in both of these high-latitude source regions occurred at approximately the same time as an abrupt increase in productivity in the EEP (Fig. 1A) and in the California margin upwelling zone (*8*), and in synchronicity with the major intensification of NHG (*29*, *30*). Recent studies suggest that polar stratification in the high-latitude source regions of EEP surface waters began at about 3 Ma (*29*, *30*). We hypothesize that nutrients that were not used at high latitudes after the intensification of NHG were instead entrained into the source waters for the EEP and fed the marked rise in productivity observed at Site 846 at ~3 Ma. The decline in productivity in the EEP between 1 and 2 Ma followed the establishment of the modern Southern Ocean opal belt at ~2 Ma (*31*), lending further support to the hypothesis that changes in nutrient availability of the source region drove major changes in EEP productivity. Given the large modern contribution of Southern Hemisphere waters to the EEP, the role that the high-latitude Southern Hemisphere climate played in the Plio-Pleistocene transition warrants more consideration.

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REPORTS

Generating Optical Schrödinger Kittens for Quantum Information Processing

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We present a detailed experimental analysis of a free-propagating light pulse prepared in a "Schrödinger kitten" state, which is defined as a quantum superposition of "classical" coherent states with small amplitudes. This kitten state is generated by subtracting one photon from a squeezed vacuum beam, and it clearly presents a negative Wigner function. The predicted influence of the experimental parameters is in excellent agreement with the experimental results. The amplitude of the coherent states can be amplified to transform our "Schrödinger kittens" into bigger Schrödinger cats, providing an essential tool for quantum information processing.

A key requirement for many quantum computation and communication protocols (*1*, *2*) is to use specific quantum states of light as a resource for information processing. We are interested in quantum states of propagating light beams, which can be analyzed either by photon counting or by homodyne detection, which measures the interference between the signal state and an intense reference beam with a relative phase θ. Homodyne detection measures a physical quantity called a "quadrature component" of the electric field, associated with the operator $\hat{x}_\theta = \hat{x}\cos\theta + \hat{p}\sin\theta$, where $\hat{x}$ and $\hat{p}$ are canonically conjugate field observables. The operators $\hat{x}$ and $\hat{p}$ are analogous to the position and the momentum of a particle, and they are often called quantum continuous variables (QCVs). From Heisenberg's inequalities, they cannot be determined simultaneously with an infinite precision, so one cannot generally define a proper phase-space density $\Pi(x, p)$ for the electric field. However, one can define a quasi-distribution $W(x, p)$ called the Wigner function, the marginals of which yield the probability distributions $P(x_\theta)$. By measuring the distributions $P(x_\theta)$ for several values of θ, one can reconstruct the Wigner function; this inverse process is known as quantum tomography (*3*).

For specific quantum states, the Wigner function can take negative values, thereby excluding any description by a classical phase-space den-

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sity. Generating such states for propagating light beams is of special interest for QCV information processing, because it provides the basis for entanglement distillation (*4*–*6*), universal quantum computing (*7*–*9*), and proposed loophole-free tests of Bell's inequalities (*10*, *11*). Such states have been realized recently by combining homodyne detection with photon counting, so that the quadrature components are measured only when a photon is detected in another (triggering) channel (*12*–*16*). We have shown recently that one can "de-Gaussify" a squeezed state of the light and turn it into a state with a Wigner function that was observed to be strongly non-Gaussian, although still positive (*14*, *17*, *18*). In the experiment presented here, we observed a propagating light field that exhibits a negative-valued Wigner function and is very close to a small Schrödinger cat state.

Among nonclassical states, Schrödinger cat states play an especially interesting role. Defined as quantum superpositions of classical distinguishable states, they are very useful to study the process of decoherence involved in the transition from quantum to classical physics (*19*), which strongly limits the development of quantum computing and communications. Coherent states are the most "classical" available in quantum physics, and we will call a Schrödinger cat state a quantum superposition of coherent states, well separated in phase space, for example $|\psi\rangle = c(|\alpha\rangle - |-\alpha\rangle)$, where $|\alpha|$ is the amplitude of the coherent states and c is a normalization factor. In this case, $|\alpha|^2$ defines the size of the cat, which becomes a "Schrödinger kitten" when $|\alpha|^2$ is small. The Wigner function of such a state presents a negative value at the origin. We wish to generate free-propagating optical Schrödinger kittens, which, unlike Schrödinger cats generated in cavities or bound systems (*19*–*21*), can be used for quantum communications. Such kittens can be produced with a very high fidelity, either by subtracting one photon from a squeezed vacuum state (*22*) (Fig. 1) or by squeezing one photon (*8*, *23*). The subtraction procedure, which is simpler to implement, can be realized by reflecting toward an avalanche photodiode (APD) a small fraction of a squeezed vacuum beam produced by a degenerate optical parametric amplifier. The APD will herald the subtraction of at least one photon, and the probability to subtract more than one photon will become negligible for a low reflectivity. Thus, an APD detection will project the transmitted beam into the desired state.

In our experiment (Fig. 2), a squeezed vacuum beam is produced in a frequency-degenerate optical parametric amplifier (DOPA) by down-conversion of frequency-doubled femtosecond laser pulses (*24*). A beamsplitter reflects 9.5% of the squeezed beam toward an APD through a filtering system, whereas the transmitted beam is analyzed by a homodyne detection. This detection works in a time-resolved regime and measures one quadrature $\hat{x}_\theta$ for each incoming pulse.

Quantum superpositions are very fragile, and four main sources of decoherence appear in our case. First, the state conditioned by the APD must belong to the spatio-temporal mode analyzed by the homodyne detection. We will consider that it actually belongs to this mode with a probability ξ and to an orthogonal mode with a probability $1 - \xi$. This modal purity parameter ξ is decreased by the limited spatial and spectral quality of the optical beams, by the imperfections of the filtering system, and by the APD dark counts. These effects mix the ideal photon-subtracted state with a nonconditioned squeezed state. The experiment is also extremely sensitive to excess noise from the DOPA, which can be modeled by introducing a

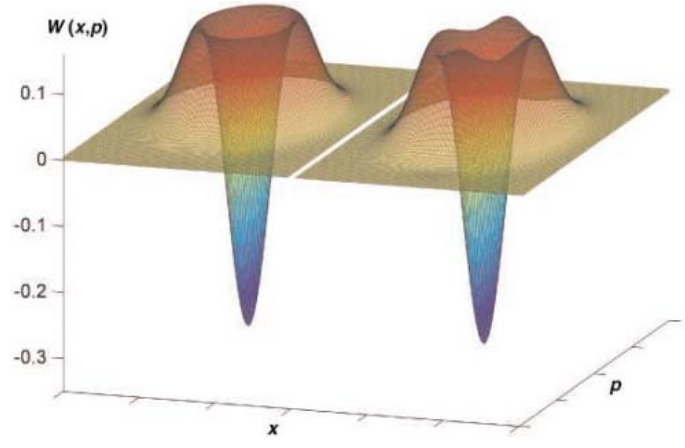

Fig. 1. Wigner function of an ideal photon-subtracted squeezed state (s = 0.6) (**left**), compared with the one of the closest Schrödinger kitten ($|\alpha_{opt}|^2$ = 0.8) (**right**). For any value of $|\alpha|^2 < 1$, the theoretical fidelity between the two matched states is larger than 0.997.

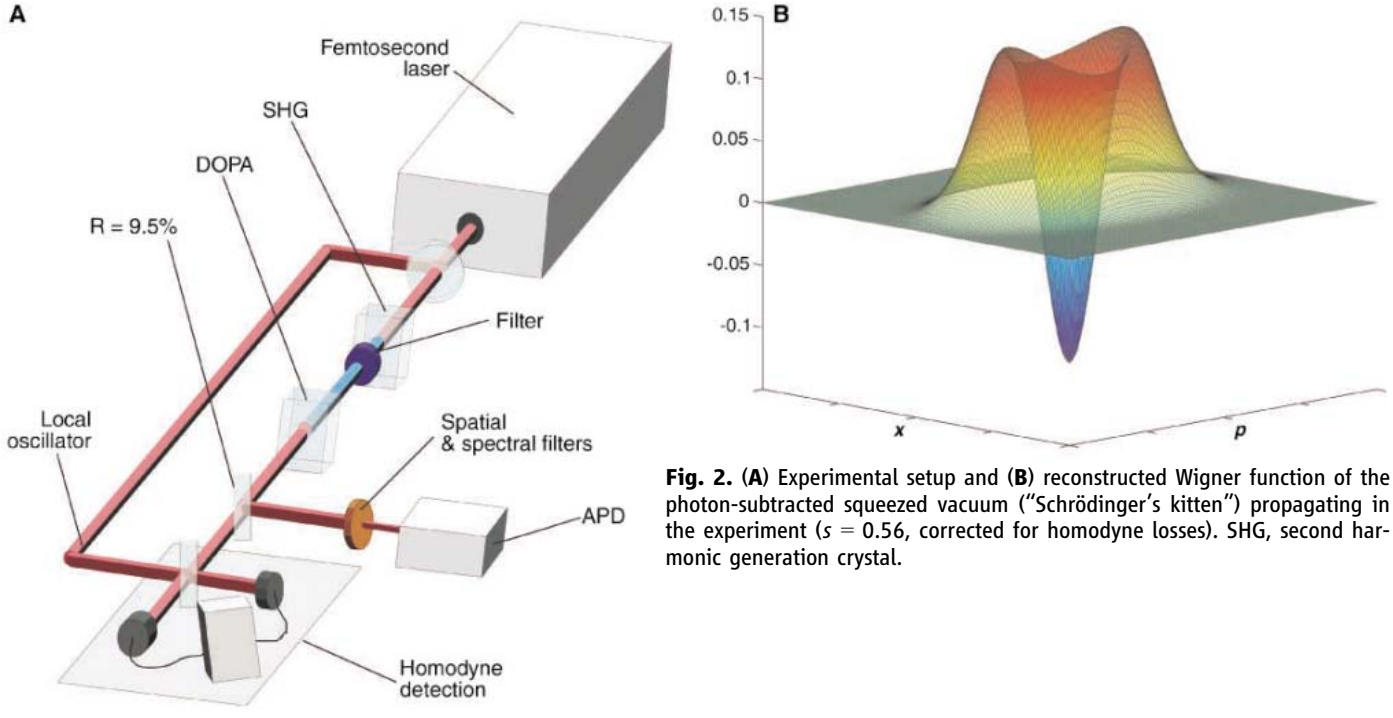

Fig. 2. (**A**) Experimental setup and (**B**) reconstructed Wigner function of the photon-subtracted squeezed vacuum ("Schrödinger's kitten") propagating in the experiment (s = 0.56, corrected for homodyne losses). SHG, second harmonic generation crystal.

second, phase-independent optical amplifier with a gain $h = \cosh^2(\gamma r_s)$ after an ideal degenerate amplifier squeezing the quantum noise variance by a squeezing factor $s = \exp(-2r_s)$, where γ is the ratio between the two amplification efficiencies, γr_s and r_s. A nonzero γ adds uncorrelated APD counts and homodyne noise. Furthermore, the reflectivity R of the pickup beamsplitter has to be large enough to ensure a count rate much above the number of dark counts of the APD. Therefore, it introduces finite losses on the transmitted beam, mixing it with vacuum. Finally, two defects appear on the homodyne detection side: the limited homodyne detection efficiency η, and the electronic noise e (normalized to the shot-noise value). However, these defects are not involved in the generation but only in the characterization of the state. They can be estimated independently and corrected for in order to determine the Wigner function of the prepared state. All of these parameters are extremely critical, and every percent loss strongly deteriorates the state. Generating states with negative Wigner functions required carefully adjusting the filtering system and special attention to the wavefront quality of the beams, in particular during the extraction of the pulses from the laser cavity and during the frequency-doubling process. An optimized homodyne detection design allowed us to observe negative values without correcting for detection losses (*24*).

We show in (*24*) that in realistic and quite general experimental conditions, the Wigner function and the marginal distributions of the detected state have very simple analytic expressions that are parameterized by four quantities—$\sigma_1(s)$, $\sigma_1(1/s)$, $\sigma_2(s)$, and $\sigma_2(1/s)$—which are functions of the experimental parameters introduced above. This model allows not only for theoretical predictions, but also for a very efficient data analysis, because these quantities can be extracted from the second and fourth moments of the measured distributions $P_{exp}(x_\theta)$ with a simple algebraic procedure.

We generated and characterized three kittens of different sizes defined by squeezing factors $s_1 = 0.56$, $s_2 = 0.60$, and $s_3 = 0.63$. For each kitten, a quantum tomography was performed by measuring six quadrature distributions $P_{exp}(x_\theta)$ for $0 \le \theta \le 5\pi/6$. For each value of θ, approximately 20,000 experimental points were acquired and divided into 64-bin histograms. The analysis procedure described above provides, without any free parameter, an excellent fit to the data (Fig. 3), as well as a simple analytical expression for the (raw data) Wigner function. The Wigner function reconstructed using this method is in very good agreement with the one obtained from the model-independent Radon transform (Fig. 3) applied to the uncorrected experimental data. In addition, our procedure provides values for all experimental parameters, which are found to be fully consistent with the values directly measured during the experiment (Table 1).

It is clear from Fig. 3 that these measurements provide a negative value for the uncorrected Wigner function, $W_{raw}(0) = -0.026 \pm 0.012$, whereas this was not the case in the experiments described in (*14*). The quality of the above fits gives us confidence that the experimental parameters provided by our analysis procedure are correct. In order to determine the Wigner function of the generated state, we have to correct for the effect of a limited homodyne detection efficiency η and an excess noise e. This can be done by the standard maximal-likelihood method (*25*, *26*), but we checked that the very same results are obtained immediately from our model; we simply replace the values of η and e determined from our data by $\eta = 1$ and $e = 0$ in the expression of the Wigner function. All the other parameters, which are involved in the generation process, are kept unchanged. The resulting Wigner function for $s = 0.56$ is displayed in Fig. 2, showing a very strong negativity, $W_{cor}(0) = -0.13 \pm 0.01$. This is to be compared with -0.32 for a perfect setup with an infinitely low beamsplitter reflectivity, and -0.25 for a reflectivity of 9.5% and $s = 0.56$.

The reconstructed state is similar to a Schrödinger kitten of size $|\alpha|^2 = 0.79$, with a fidelity $F_{cat} = 70\%$. Clearly it is still a statistical mixture, mostly because of the conditioning process, which is not corrected here because it is involved in the generation of the state. It can thus be decomposed further in pure states, and

Table 1. Analysis of the generated states, after correction for the homodyne efficiency $\eta = 80\%$ and electronic noise $e < 0.04$ shot-noise units. There is no correction for the excess noise factor γ and the modal purity ξ (the values presented below are directly obtained from the fitting procedure). Here s is the squeezing factor, $W(0)$ is the Wigner function negativity, F_{vac} is the fraction of initial squeezed state, and F_{cat} is the fidelity with the most similar Schrödinger kitten of size $|\alpha_{opt}|^2$. As expected, in this range of parameters, the size of the kitten increases when the initial state is more and more squeezed (smaller s).

s	$W(0)$	F_{vac}	F_{cat}	$\lvert\alpha_{opt}\rvert^2$	γ	ξ
0.56	-0.13 ± 0.01	29%	70%	0.79	0.17	0.82
0.60	-0.09 ± 0.01	35%	64%	0.71	0.47	0.89
0.63	-0.08 ± 0.01	36%	63%	0.62	0.45	0.86

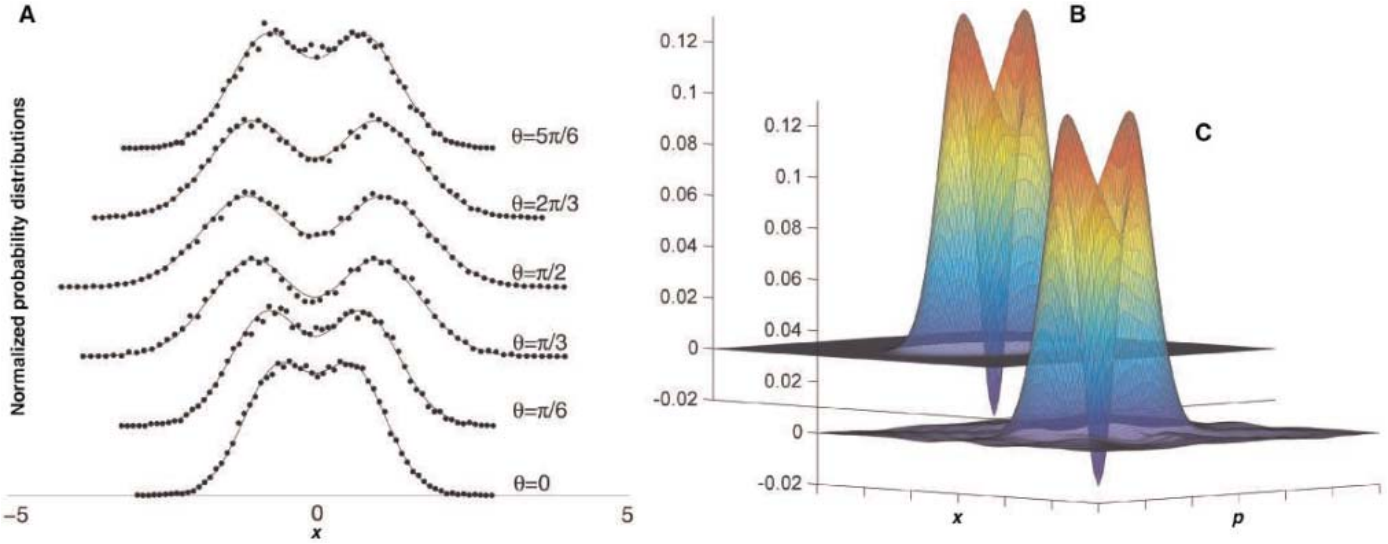

Fig. 3. (**A**) Experimentally measured quadratures and theoretical fits, corresponding to a squeezing factor $s = 0.56$. (**B**) Wigner function obtained by the generic model (*24*). (**C**) Wigner function obtained by Radon transform of the raw experimental data. In all of these curves, no correction was made for detection efficiencies, which are included in the generic model together with other imperfections.

simple calculations show that it can be written as 70% of the ideal photon-subtracted state, 29% of the initial squeezed state, and 1% of residuals. If our state was a purely statistical mixture of coherent states, this fidelity would be 0.40 and would remain below 0.50 for any $|\alpha|^2$. The results for the various experimental values of s are presented in Table 1.

The present procedure allows the reproduction of small Schrödinger kittens, but the structure of a larger cat becomes more complicated (*24*). For "growing the cat," the generated kitten's state should be taken as a starting point to initiate a "breeding" process as described by Lund *et al*. (*23*). This can be done by combining two kitten states of a beamsplitter and using one output channel for another photon-counting "purifying" measurement, whereas the other channel provides the cat state with a larger amplitude. Because the quality of our state is actually higher than it was assumed in (*23*), such an experiment can be realistically envisioned. Our setup also allows, with minor modifications, the generation of quadrature-entangled pulses (*27*). Subtracting one photon from each mode provides entangled beams with negative Wigner functions, which have been proposed to improve the fidelity of continuous-variable teleportation (*28*–*30*) and to implement a loophole-free Bell test (*10*, *11*). These examples clearly show that the availability of the states demonstrated here opens the way to many new quantum information and communication protocols.

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Reactive and Nonreactive Scattering of H_2 from a Metal Surface Is Electronically Adiabatic

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The Born-Oppenheimer approximation of uncoupled electronic and nuclear motion is a standard tool of the computational chemist. However, its validity for molecule–metal surface reactions, which are important to heterogeneous catalysis, has been questioned because of the possibility of electron-hole pair excitations. We have performed experiments and calculations on the scattering of molecular hydrogen from a catalytically relevant metal surface, obtaining absolute probabilities for changes in the molecule's velocity parallel to the representative Pt(111) surface. The comparison for in-plane and out-of-plane scattering and results for dissociative chemisorption in the same system show that for hydrogen-metal systems, reaction and diffractive scattering can be accurately described using the Born-Oppenheimer approximation.

Theoretical chemistry has been very successful at making predictions regarding gas-phase reactions (*1*–*4*) and scattering (*5*). For the simplest reaction of H with H_2, theorists can provide very detailed interpretations. For instance, they can determine the mechanisms giving rise to the formation of short-lived collision complexes, which are known experimentally to affect the quantum-state resolved angular product distributions (*3*, *4*).

In the theoretical description of chemical reactions, the Born-Oppenheimer (BO) approximation (*6*) is the standard tool of the computational chemist. Transition-state theory, used to compute reaction rates for complex systems, depends on the validity of this approximation (*7*). The BO approximation successfully describes many gas-phase reactions (*1*), although treatments of reaction (*2*) and scattering (*5*) going beyond this approximation are necessary in specific cases. In the electronically adiabatic treatment implied by the BO approximation, the electrons adjust instantaneously to the movement of the nuclei. An advantage of the BO approximation is that it allows dynamics results for reactive scattering to be computed in a straightforward way, in two steps. First, the electronic Schrödinger equation is solved with the nuclei frozen to obtain a potential energy surface (PES) specifying the forces between the atoms. Next, the PES is used in the Schrödinger equation for nuclear motion to obtain reaction and scattering probabilities.

It would be of considerable use if the same simple theoretical approach could be taken to the reactive scattering of molecules from metal surfaces. This class of problems is important to the field of heterogeneous catalysis, which is of tremendous practical interest: About 90% of the chemical manufacturing processes employed throughout the world use catalysts in one form or another (*8*). However, the ability of electronically adiabatic theory to describe the reactive scattering of molecules from metal surfaces is currently a topic of intense debate. In a metal surface, electronic excitations, also called electron-hole (e-h) pair excitations, can take

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place with infinitesimally small excitation energies. These could act as an energy sink or source and thereby affect a molecule's reaction on or scattering from a metal surface. Direct evidence that e-h pair excitations can accompany molecule-surface scattering comes from recent observations of electronic excitation after highly exothermic chemisorption of atoms and molecules (*9*), and of electron emission after collisions of highly vibrationally excited NO molecules with a low-work-function metal surface (*10*). The first process should be relevant to molecule-surface reactions exhibiting deep chemisorption wells in front of the barrier to reaction, whereas the second process should be important for molecules with high electron affinity.

The above observations raise the questions of whether the BO approximation is valid for any particular class of molecule–metal surface reactions and how to test for such validity. The validity of the BO approximation for a molecule–metal surface reaction is best tested by comparison of electronically adiabatic theory to detailed experiments on both reaction and scattering. The comparison for reaction probes the presence of energy dissipation to e-h pairs that accompanies the molecule's motion toward the reaction barrier. The comparison for scattering tests the presence of energy dissipation both on the way to and on the way back from the barrier. It is conceivable that an electronically adiabatic theory could reproduce experiment for reaction due to the fortuitous cancellation of errors in the PES and errors resulting from the neglect of nonadiabatic effects. However, it is extremely unlikely that adiabatic theory could reproduce both reaction and scattering experiments in the presence of significant nonadiabatic energy dissipation.

Here we show that theory can accurately describe both reaction and diffractive scattering (*11*) in H_2–metal surface systems within an electronically adiabatic picture. This study takes advantage of recent detailed experimental data on diffractive scattering of H_2 from Pt(111) and of an adiabatic PES that accurately describes the corrugation of the H_2-Pt(111) interaction (*12*) as obtained from density functional theory (DFT). Specifically, we present absolute in-plane and out-of-plane diffraction probabilities for nine transitions and a large range of incidence energies (E_i's) for the two main symmetry incidence directions of the surface. Comparison of these probabilities with new dynamics results regarding diffraction, together with the present and previous (*13*, *14*) results for dissociative chemisorption of H_2 on Pt(111) at normal and off-normal incidence, shows that e-h pair excitations do not affect the reactive and nonreactive scattering to a significant extent. A system consisting of H_2 plus a metal surface was selected because these systems are important in a wide range of catalytic processes as well as in hydrogen storage (*8*). This class of systems is also relevant as a benchmark: If the H + H_2 reaction (*3*, *4*) is the simplest gas-phase reaction then, by analogy, the simplest molecule-surface reaction is the dissociative chemisorption of H_2 on a metal surface (*15*, *16*).

The diffraction experiments (*17*) were performed for fixed angles of incidence θ_i (Fig. 1A) measuring both in-plane and out-of-plane diffraction, as defined by the angle ϕ (Fig. 1A). Experimental results were obtained for incidence along the two main symmetry directions of the Pt(111) surface (Fig. 1, B and C) in the form of angular distributions for in-plane and out-of-plane scattering (Fig. 1, D and E). The incident beam's intensity was measured and used to normalize scattered beam intensities with respect to the incident beam, thereby yielding absolute diffraction probabilities. The scattering experiments covered the range of collision energies (up to 15 kJ/mol) relevant to heterogeneous catalysis: The average collision energy of H_2 with a Pt surface is 2 kT = 12.5 kJ/mol for the most important catalytic process involving H_2 and Pt, the reforming of gasoline, which proceeds at 750 K (*8*). The quantum-dynamical calculations (*17*) modeled the motion in all six degrees of freedom of H_2 (Fig. 2A) and were based on a DFT PES (*17*). A two-dimensional cut through the PES used is shown in Fig. 2B, and one-dimensional cuts are shown in Fig. 2C.

Absolute diffraction probabilities extracted from the measured angular distributions (Fig. 1, D and E) are compared to theoretical results for an initial translational energy of H_2 parallel to the surface (E_{par}) of 5.3 kJ/mol in Fig. 3. The agreement between theory and experiment is very good. The energy dependence and the relative values of the diffraction probabilities are well reproduced by the theory. In particular, for the [11$\bar{2}$] incidence direction, the theory reproduces the following features: (i) the predominance of specular scattering, the probability of which [P(0,0)] is larger than that of first-order in-plane diffraction [(P(–1,–1) + P(1,1)] and also larger than the sums of first-order sideways backward diffraction probabilities [P(–1,0) + P(0,–1)] and of first-order sideways forward diffraction probabilities [P(1,0) + P(0,1)]; (ii) the order in size of P(0,0), [P(–1,0) + P(0,–1)], [P(1,0) + P(0,1)], and P(–1,–1) at low incidence energies; and (iii) the similar size of [P(–1,0) +

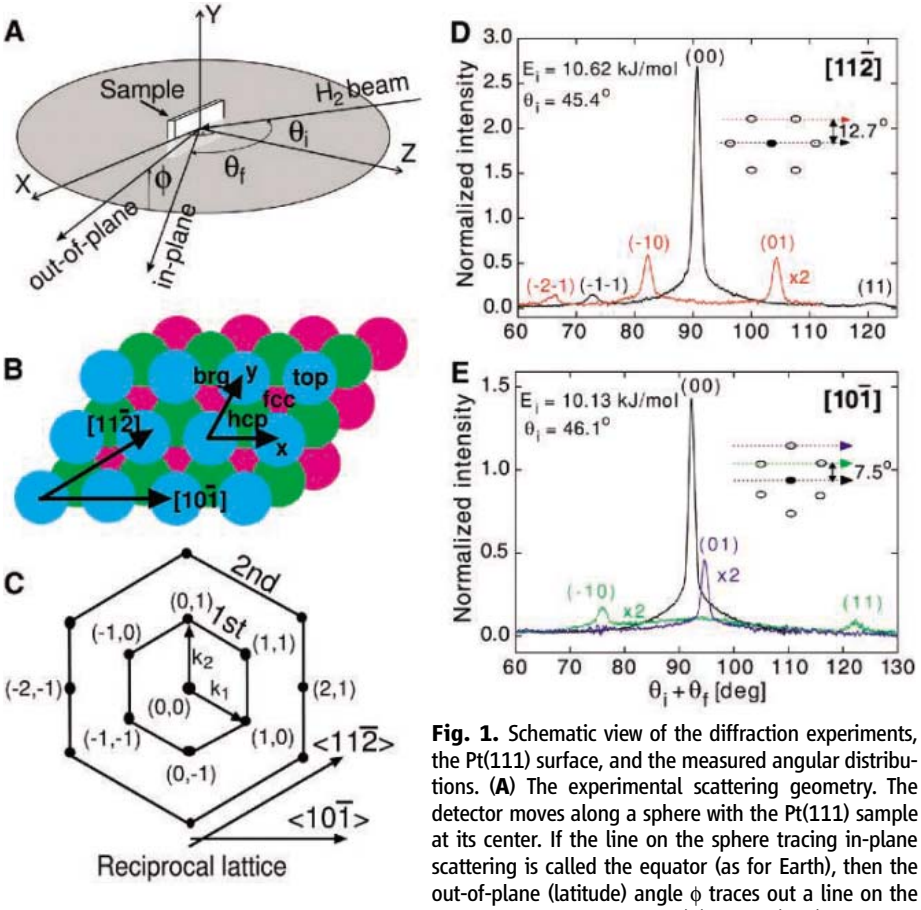

Fig. 1. Schematic view of the diffraction experiments, the Pt(111) surface, and the measured angular distributions. (**A**) The experimental scattering geometry. The detector moves along a sphere with the Pt(111) sample at its center. If the line on the sphere tracing in-plane scattering is called the equator (as for Earth), then the out-of-plane (latitude) angle ϕ traces out a line on the sphere along which out-of-plane diffractive transitions may be measured. (**B**) The Pt(111) surface is shown in real space, along with the surface unit cell spanned by x and y; the position of the high-symmetry sites top, bridge (brg), face-centered cubic (fcc) hollow, and hexagonal close-packed (hcp) hollow; and the [11$\bar{2}$] and [10$\bar{1}$] incidence directions. (**C**) The corresponding reciprocal lattice, with labeling of the diffraction transitions (n,m). (**D** and **E**) Angular distributions (given in units of 10^{-2} of the incident beam's intensity) from experiments with the H_2 beam incident along the [11$\bar{2}$] and the [10$\bar{1}$] directions, respectively. The surface temperature was 463 K. Black curves are for in-plane scattering and colored curves are for out-of-plane scattering. The angles Θ_i, Θ_f, and ϕ are defined in (**A**).

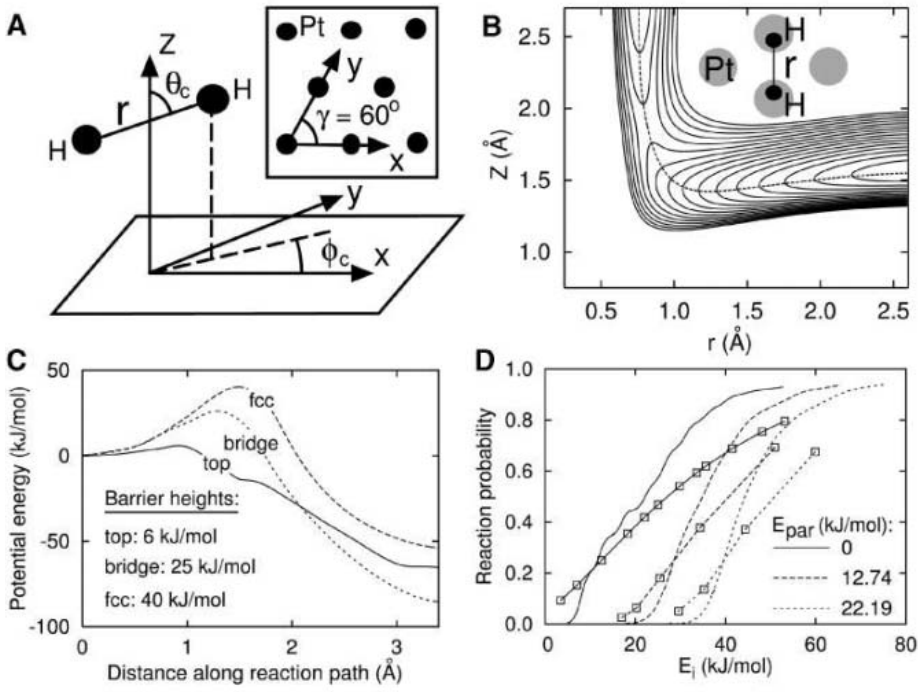

Fig. 2. Coordinate system and cuts through the PES used in the calculations, and a comparison between theoretical and experimental reaction probabilities. (**A**) The coordinate system used in the dynamics calculations. (**B**) A two-dimensional cut through the H_2 + Pt(111) PES used in the calculations, for the dissociation geometry indicated (that is, for dissociation above a bridge site with the molecule held parallel to the surface). *Z*, the molecule-surface distance; *r*, the H-H distance. Contour lines are drawn at intervals of 10 kJ/mol, with 0 kJ/mol corresponding to the H_2 gas-phase minimum. (**C**) The potential energy along the minimum-energy path for dissociation for three (fixed) high-symmetry impact sites. The barrier heights associated with the paths are also indicated. (**D**) Theoretical reaction probabilities computed for normal and off-normal incidence along the [11$\bar{2}$] direction compared to experimental results (squares) (*13*).

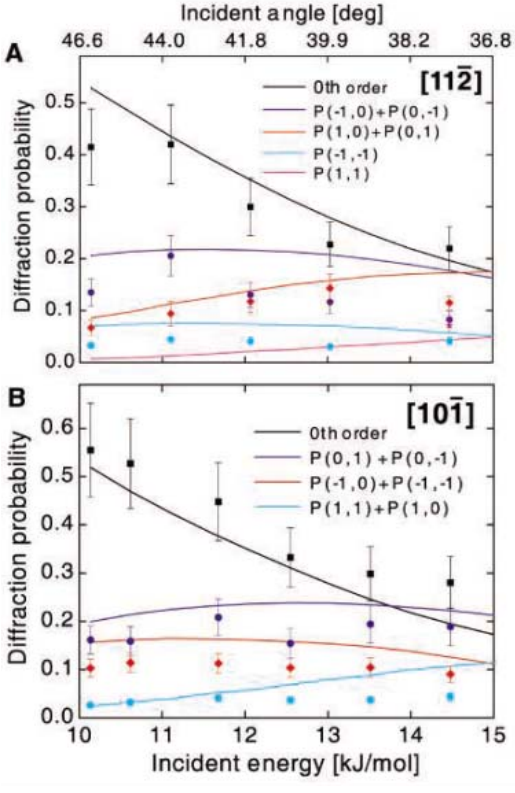

Fig. 3. (**A**) Experimentally determined diffraction probabilities (symbols) compared with computed diffraction probabilities (curves) for specular scattering (black) and several first-order out-of-plane (dark blue and orange) and in-plane (light blue and pink) diffractive scattering transitions. The results are for incidence along the [11$\bar{2}$] direction. (**B**) Results for incidence along the [10$\bar{1}$] direction for specular scattering (black) and several first-order out-of-plane (colored symbols and curves) diffractive scattering transitions. Probabilities for symmetry equivalent transitions were summed. Error bars represent 68% confidence intervals.

P(0,–1)] and [*P*(1,0) + *P*(0,1)] on the one hand, and of *P*(–1,–1) and *P*(1,1) on the other hand, at higher energies. The agreement for the [10$\bar{1}$] incidence direction is likewise very good: The order in the size of *P*(0,0), [*P*(0,1) + *P*(0,–1)], [*P*(–1,0) + *P*(–1,–1)], and [*P*(1,1) + *P*(1,0)] is correctly described except at the highest incidence energy used experimentally.

We emphasize at this point the importance of our experimental and theoretical treatment of both in-plane and out-of-plane diffraction. It is only in this way that the full extent of agreement between experiment and theory can be established. This complete treatment also nicely solves a long-standing paradox regarding the corrugation of Pt(111) as seen by H_2 (*18*). Evidence for a large corrugation had previously been found in experiments on reaction, which showed a large dependence of the reaction probability on E_{par} (*13*). However, the flatness of the Pt(111) surface was conversely supported by scattering experiments for E_i = 10.7 kJ/mol and the same E_{par} as used in our work, which only considered scattering of H_2 in the plane of incidence (*19*). Those experiments found small probabilities for in-plane diffraction relative to specular scattering, as we did [note the small values of *P*(–1,–1) and *P*(1,1) in Fig. 3A]. However, no attempt was made to observe out-of-plane scattering (*19*). The present experiments show that, in scattering, the large corrugation of the surface manifests itself through large probabilities for out-of-plane diffraction, for reasons concerning the dynamics (*14*, *20*).

We now proceed to reaction probabilities computed for the [11$\bar{2}$] incidence direction, which are compared to experimental reaction probabilities (*13*) in Fig. 2D, for normal and off-normal incidence with an E_{par} of 12.7 and 22.2 kJ/mol. Although the experimental results are for rotationally warm molecular beams, the theoretical results for normal incidence and for E_{par} = 12.7 kJ/mol are for cold n-H_2 (25% j = 0 and 75% j = 1, where j is the angular momentum quantum number of H_2), and the results for E_{par} = 22.2 kJ/mol were obtained for j = 0 H_2 only. These approximations are justified in supporting online text and in fig. S2. The experimental reaction probabilities for off-normal incidence were obtained by interpolating experimental results at fixed incidence angles but variable incidence energy to results for fixed E_{par} and variable total incidence energy. Excellent agreement is obtained for both normal and off-normal incidence, as was found before for normal incidence and for two different values of E_{par}, modeling hydrogen by cold p-H_2 (j = 0 H_2) in the calculations (*14*). In particular, the variation of the reaction threshold with E_{par} is well described and there is good overall quantitative agreement, although the theoretical reaction probability curves are somewhat too narrow, as analyzed elsewhere (*21*) in detail (see also supporting online text).

The above comparisons (Figs. 2D and 3) show that electronically adiabatic theory can

provide a quantitatively accurate description of both reactive and diffractive scattering of H_2 from metal surfaces. Other research has already shown that electronically adiabatic dynamics calculations can also yield a good description of rotationally inelastic scattering of H_2 from a metal surface (*22*) if surface motion is modeled explicitly (*23*). An inspection of Fig. 2, B and C, and Fig. 4 helps explain why electronically adiabatic theory is so successful in describing hydrogen's reaction on and scattering from metal surfaces. The DFT potential for H_2 on Pt(111) shows no deep chemisorption well between the reactants region and the reaction barrier. This result is general for H_2 + metal systems (*16*), which only show shallow physisorption wells, and suggests that e-h pair excitations, which occur to a significant extent in systems with a deep chemisorption well (*9*), should not have an important influence on the scattering and on whether or not H_2 reacts. Also, H_2 has a negative electron affinity, so that the molecule would accept a free electron moving in the gas phase only for bond extensions larger than 1.6 Å [Fig. 4A and (*24*)]. This property rules out a strong influence of nonadiabatic effects seen in metal surface scattering of molecules with high electron affinity, such as efficient vibrational de-excitation of (v = 2, where v is the vibrational quantum number) NO (*25*) and efficient multiquantum vibrational relaxation of (v = 15) NO incident on Au(111) (*26*), as well as electron ejection in scattering of highly vibrationally excited NO from Cs/Au(111) (*10*). Specifically, vibrationally excited NO can drive electronic excitation by accepting an electron from a metal surface at large bond extensions and transferring back the charge at small bond extensions (Fig. 4B), because the electron transfer will not generally occur instantaneously when the potential curves for NO + e^- and NO^- cross (*26*). Qualitative nonadiabatic models (*27*) and more elaborate, but still approximate, theories (*28*) suggest that e-h pair excitations in H_2–metal surface scattering are small because of the short lifetime $\tau = \hbar/2\Delta$ of the hole associated with the antibonding Σ_u H_2 level [which has acquired a large width Δ once it drops below the Fermi level (*27*)] and the relatively small charge rearrangement associated with the breaking of a single σ bond (*28*) (see also supporting online text).

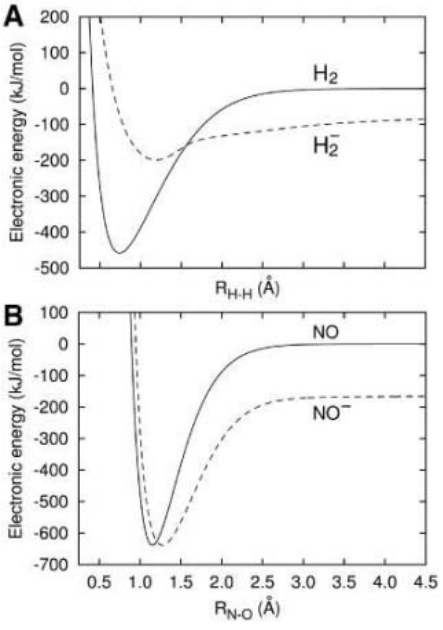

Fig. 4. (**A**) Potential energy curves for H_2 and H_2^- [from (*24*)]. *R*, the interatomic distance. Because H_2 has a relatively low affinity for electrons, electron transfer to H_2 that could drive e-h pair excitations affecting the scattering to a significant extent does not take place. (**B**) Potential energy curves for NO and NO^- [from (*26*)]. Because NO has a relatively high affinity for electrons, the potential curves cross near the equilibrium bond length of NO, allowing electron transfer to vibrationally excited NO that can drive subsequent e-h pair excitations and even electron emission in specific cases.

The most important conclusion of this work is that the application of electronically adiabatic theory to the scattering of H_2 from metal surfaces should allow the calculation of accurate reaction and diffraction probabilities. It is not that DFT, with a given generalized gradient approximation (GGA), should always provide a PES that could yield accurate results for reaction and scattering. From research on gas-phase reactions, it is well known that, with the presently used density functionals, reaction barriers computed with DFT at the GGA level can be off by 10 kJ/mol or more (*29*). As shown here and elsewhere (*14*) for H_2 + Pt(111), the use of a PES based on converged DFT calculations employing the Becke-Perdew GGA (*30*, *31*) gives reaction probability curves centered on a value of the collision energy that are in good agreement with experimental results (*13*). This shows that the average reaction barrier height was well described using the GGA employed. However, we cannot be sure at this stage that the Becke-Perdew GGA or other often-used GGAs will provide accurate PESs for other H_2–metal surface systems. Recently, it was shown that reaction probability curves calculated with two widely used GGAs [(PW91 (*32*) and RPBE (*33*)] for H_2 + Ru(0001) were shifted relative to each other by more than 10 kJ/mol in collision energy. In addition, the present often-used GGAs fail to describe the attractive van der Waals energy, which may affect the calculation of diffraction probabilities (see supporting online text). However, our result that electronically adiabatic theory yields good results for reaction and diffraction if a PES is used with a correct average reaction barrier height will almost certainly stimulate further research aimed at establishing which density functional yields the best results for molecule-surface interactions and at devising better functionals.

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Cobalt-Base High-Temperature Alloys

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We have identified cobalt-base superalloys showing a high-temperature strength greater than those of conventional nickel-base superalloys. The cobalt-base alloys are strengthened by a ternary compound with the $L1_2$ structure, γ' $Co_3(Al,W)$, which precipitates in the disordered γ face-centered cubic cobalt matrix with high coherency and with high melting points. We also identified a ternary compound, γ' $Ir_3(Al,W)$, with the $L1_2$ structure, which suggests that the Co-Ir-Al-W–base systems with $\gamma + \gamma'$ $(Co,Ir)_3(Al,W)$ structures offer great promise as candidates for next-generation high-temperature materials.

Cobalt and nickel have the face-centered cubic (fcc) structure at high temperature, with melting points of 1768 and 1728 K, respectively. However, the most fascinating heat-resistant alloys are the Ni-base superalloys, which are used, for example, in aircraft engines, industrial gas turbines, reactors, and the chemical industry (*1*). The main reason why Co-base alloys have not found widespread usage is their lower strength compared with that of Ni-base alloys, but they have been studied for a long time (*2*). With the development of Ni-base superalloys strengthened by the ordered γ' $Ni_3(Al, Ti)$ phase, the possibilities of precipitation hardening using geometrically close-packed phases that have the form of A_3B have been extensively investigated. Two types of geometrically close-packed phases have been reported in Co-base alloys: Co_3Ti with the $L1_2$ structure (*3*, *4*) and ordered fcc Co_3Ta (*5*, *6*). Although the effect of various alloying elements on the stability and morphology of the γ' Co_3Ti phase has been investigated, the usefulness of the γ' phase is restricted to temperatures below 1023 K (*4*). In the case of Co_3Ta, the ordered fcc phase is metastable and readily converts to the stable hexagonal close-packed (hcp) structure Co_3Ta (*6*). Furthermore, the lattice parameter mismatches of these phases in Co-base alloys are usually more than 1.0%, which is not as useful for strengthening as the geometrically close-packed phases in Ni-base superalloys, where mismatches typically vary from –0.1 to + 0.5% (*2*). Geometrically close-packed phase strengthening has thus not been used in commercial Co-base superalloys.

In determining the phase diagram of the Co-Al-W ternary system, we found a stable ternary compound with the $L1_2$ structure, which has the form $Co_3(Al, W)$, designated as γ'. Figure 1A shows a transmission electron micrograph of Co-9Al-7.5W (atomic %) annealed at 1173 K for 72 hours after solution treatment at 1573 K for 2 hours. The cuboidal phase homogeneously precipitates in the γ(A1) matrix, which is very similar to the morphology observed in Ni-base superalloys. The selected area electron diffraction pattern of the same sample is also shown in Fig. 1B, where the crystal structure of the γ' phase is confirmed as being the $L1_2$ ordered structure and the cuboidal γ' precipitates align along the <001> directions. The compositions of the matrix and that of the precipitate were determined using a field emission electron probe microanalyzer (FE-EPMA). The composition of cuboidal precipitates observed in Fig. 1A is the ternary compound $Co_3(Al,W)$, where the composition of Al and W has an almost equiatomic ratio. In the geometrically close-packed A_3B compound, the stable Co_3W phase with the DO_{19} structure appears in the Co-W binary system (*7*). Although no stable compound of Co_3Al is formed in the Co-Al binary system, the formation of an ordered Co_3Al phase has been reported (*8*, *9*). Recently, the metastable Co_3Al phase with the $L1_2$ structure, which is formed in the Co-14Al alloy annealed at 873 K for 24 hours, was observed by our group (*10*). It can be said, therefore, that metastable Co_3Al γ' is stabilized by alloying with W. Ternary compounds of $Co_3(Al, Cr)$ or $Co_3(Al, Mo)$ have not been reported.

Figure 2, A and B, show isothermal section diagrams determined experimentally in the present study, as well as recent data (*11*) on the Co-W binary system at 1173 and 1273 K, respectively. The γ' phase is stable at 1173 K but metastable at 1273 K. The thermal stability of the γ' phase and the effect of alloying were investigated by differential scanning calorimetry (DSC). Figure 3A shows the DSC curves on heating, where the solvus temperature was determined from the endothermic peak, as indicated by arrows. The DSC curve of Waspaloy [Ni-21Cr-2.5Mo-13Co-2.9Al-3.5Ti-0.3C (atomic %)], a widely used commercial Ni-base superalloy, is also shown. The solvus temperatures of the γ' phase in the Co-Al-W ternary system is ~1263 K, which corresponds well with the phase diagram, as shown in Fig. 2. The addition of Ta stabilizes the γ' phase such that the solvus temperature is ~1373 K, and this value is higher than that of Waspaloy.

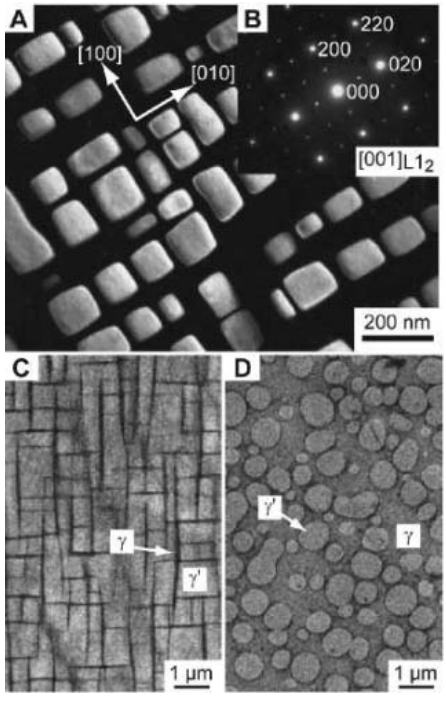

Fig. 1. Electron micrographs of Co-9Al-7.5W alloy annealed at 1173 K for 72 hours. (**A**) Dark-field image. (**B**) Selected area diffraction pattern. (**C** and **D**) Field emission scanning electron micrographs of Co-8.8Al-9.8W-2Ta (C) and Co-8.8Al-9.8W-2Mo (D) annealed at 1273 K for 1 week.

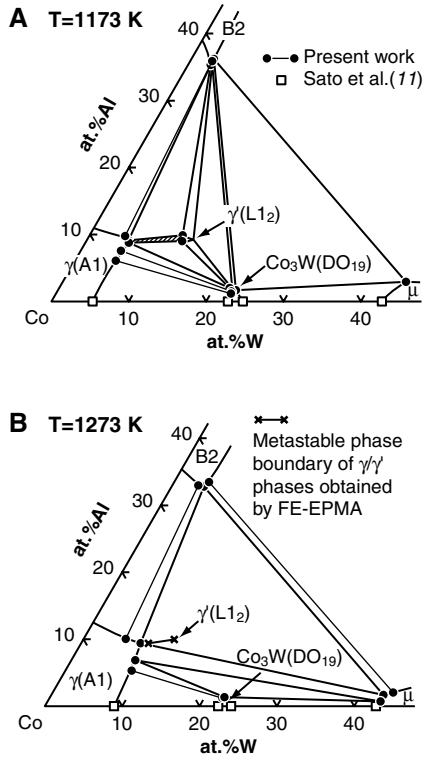

Fig. 2. Isothermal section diagrams of the Co-Al-W ternary system in the Co-rich portion at (**A**) 1173 K and (**B**) 1273 K.

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The addition of Nb or Ti shows a similar effect. It can also be seen from Fig. 3A that the melting temperatures of Co-Al-W–base alloys are ~1673 K, which is 50 to 100 K higher than those of Ni-base superalloys (*12*). Figure 3B shows the temperature variation of Vickers hardness of the $\gamma + \gamma'$ structure for Co-9.2Al-9W and Co-8.8Al-9.8W-2Ta aged at 1073 K for 24 hours after solution treatment at 1573 K for 2 hours. Aging treatment of Waspaloy was carried out at 1118 K for 24 hours and 1033 K for 16 hours after solution treatment at 1353 K for 4 hours. The hardness of the $\gamma + \gamma'$ structure of the Co-9.2Al-9W alloy is very similar to that of Waspaloy. The addition of Ta increases the hardness, which might be due to the stabilization of the γ' phase up to 1373 K. The 0.2% compressive proof strengths of Co-9.2Al-9W and Co-8.8Al-9.8W-2Ta alloys are 473 and 674 MPa at 1143 K, respectively, as compared with 520 MPa for the 0.2% tensile proof strength of Waspaloy (*12*). These data correspond well with the results for high-temperature hardness in Fig. 3B.

Semiquantitative analyses of the partition behavior of various alloying elements between the γ and γ' phase were also carried out by EPMA. The results show that Mo, Ti, Nb, V, and Ta distribute to the γ' phase rather than the γ phase and stabilize the γ' phase, whereas Fe, Mn, and Cr tend to distribute to the γ phase, which is similar to the case of Ni-base superalloys (*13*, *14*). It is notable that Ni is almost equally distributed in the γ and γ' phases and substitutes for more than 50% of the Co with the rise in the solvus temperature of the γ' phase. For instance, the γ' solvus temperature of Co-20Ni-10Al-10W-2Ta is ~1423 K. Because the partition coefficient between the γ and γ' phases depends on the composition and temperature, as in the case of Ni-base superalloys (*13*), more systematic studies are required.

The lattice parameters of the γ and γ' phases of Co-9.2Al-9W alloy heat-treated at 1173 K were determined by room temperature x-ray diffraction at 0.3580 and 0.3599 nm, respectively. The lattice parameter mismatch is thus 0.53%, which is similar to that of Ni-base superalloys. The lattice parameter mismatch affects the morphology of the precipitate, because the mismatch is the driving force in the growth and coalescence of γ' particles. Figure 1, C and D, show field emission electron scanning micrographs of a typical $\gamma + \gamma'$ structure of Co-8.8Al-9.8W–base alloy annealed at 1273 K, where the addition of 2 atomic % Ta and Mo changes the morphology and the volume fraction of the γ' phase. The volume fraction of the γ' phase is increased by the addition of Ta, which is due to the increase in solvus temperature, as shown in Fig. 3. The spherical γ' phase shown in Fig. 1D suggests that the γ/γ' interface is coherent and stable. These findings suggest that the alloy design of Co-Al-W–base superalloys can be achieved under a wide variety of conditions, as is the case for the Ni-base superalloys.

The present Co-Al-W alloys exhibit very good hot workability. Because the melting temperatures of Co-Al-W–base alloys are higher than those of conventional Ni-base superalloys (Fig. 3), hot-working could be carried out in a wider temperature range than was possible in the Ni-base alloys, although new Ru-containing nickel superalloys with higher melting temperatures have been reported (*15*).

The $\gamma + \gamma'$ structure also shows good mechanical properties at room temperature. The tensile properties of Co-9.2Al-9W heat-treated at 1173 K for 1 hour after hot-rolling are as follows: 0.2% proof and tensile strengths of 737 and 1090 MPa, respectively, with 20% elongation; these are comparable with the tensile properties of Ni-base superalloys such as Waspaloy, with 0.2% proof and tensile strengths of 795 and 1275 MPa, respectively, and 25% elongation (*12*).

We note a ternary compound, $\gamma' Ir_3(Al,W)$, with the $L1_2$ structure. Figure 4 shows the electron micrograph of a dark-field image and the selected area diffraction pattern of Ir-10Al-10W alloy annealed at 1573 K for 72 hours, which confirms that the γ' phase with the $L1_2$ structure finely precipitates. The high-temperature hardness of Ir-10Al-10W and Ir-20Co-10Al-10W alloys annealed at 1573 K for 24 hours is shown in Fig. 3B, where high hardness is maintained even at 1273 K. Because Ir has a melting temperature of 2720 K and the fcc γ phase in the Co-Ir binary system shows a complete solid solution, the Ir-Al-W and Co-Ir-Al-W–base systems with $\gamma + \gamma'$ $(Co,Ir)_3(Al,W)$ structures offer great promise as candidates for next-generation high-temperature materials (*16*).

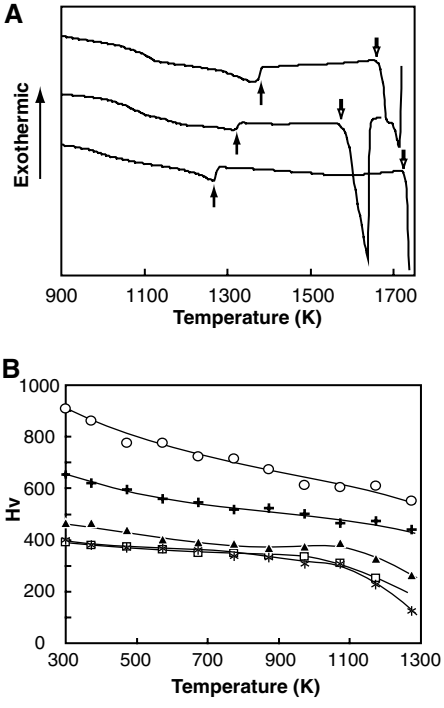

Fig. 3. (**A**) DSC curves. (Top) Co-8.8Al-9.8W-2Ta; (middle) Waspaloy; (bottom) Co-9.2Al-9W. (**B**) High-temperature Vickers hardness. (○) Ir-20Co-10Al-10W, (+) Ir-10Al-10W, (*) Co-9.2Al-9W, (▲) Co-8.8Al-9.8W-2Ta, (□) Waspaloy.

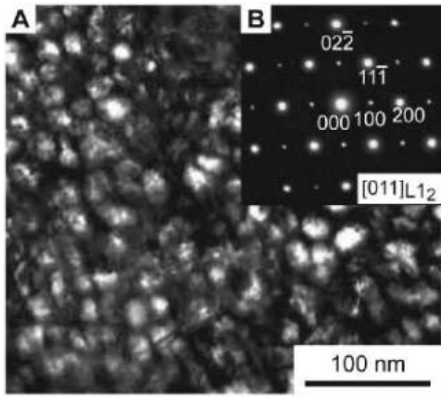

Fig. 4. Electron micrographs of Ir-10Al-10W alloy annealed at 1573 K for 72 hours. (**A**) Dark-field image. (**B**) Selected area diffraction pattern.

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New Dust Belts of Uranus: One Ring, Two Ring, Red Ring, Blue Ring

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We compared near-infrared observations of the recently discovered outer rings of Uranus with Hubble Space Telescope results. We find that the inner ring, R/2003 U 2, is red, whereas the outer ring, R/2003 U 1, is very blue. Blue is an unusual color for rings; Saturn's enigmatic E ring is the only other known example. By analogy to the E ring, R/2003 U 1 is probably produced by impacts into the embedded moon Mab, which apparently orbits at a location where nongravitational perturbations favor the survival and spreading of submicron-sized dust. R/2003 U 2 more closely resembles Saturn's G ring, which is red, a typical color for dusty rings.

Showalter and Lissauer (*1*) reported the detection of two faint rings, R/2003 U 1 (R1) and R/2003 U 2 (R2), outside Uranus's main ring system. R1, the outer ring, peaks at the orbit of the tiny moon Mab, whereas R2 orbits between moons Rosalind and Portia but has no visible source bodies. Using the Keck adaptive optics (AO) system at near-infrared wavelengths, we show that R2 is red, whereas R1 is extremely blue. The colors, location, and radial extent of the two rings, as well as the association of ring R1 with moon Mab, make the uranian system in many ways similar to Saturn's outer ring system.

We imaged Uranus and its ring/moon system with the near-infrared AO camera NIRC2 at the W. M. Keck II telescope on 23 August and 28 October 2005 (universal time). We observed in the K′ band (2.2 μm), where sunlight is absorbed by methane and hydrogen gas in Uranus' atmosphere, greatly reducing scattered light from the planet. The ring opening angle with respect to Earth, B, was 8.4° in August and 10.5° in October. The pixel size was ~140 km (0.01″) and the effective resolution ~650 km. The full field of view was 10″. All images were processed using standard techniques (*2*, *3*). The intensities were converted into units of I/F, where πF is the solar flux density received by Uranus at K′, and I is the reflected intensity.

R2 was detected on the planet's south side on 23 August (Fig. 1A), but only the ring's tip could be seen in the north. Both tips fell at a radial distance of 67,350 ± 100 km, consistent with Hubble Space Telescope (HST) results (*1*). In October, we mosaicked images to cover a larger field (~25″) to search for R1. We detected a portion of R2 in the south but saw no trace of the outer ring, R1, despite a clear view of both the north and south sides of this ring (Fig. 1B).

We averaged in longitude the visible parts of ring R2 to produce radial profiles (Fig. 2). These were modeled (*2*) with a uniform sheet of material that, after convolution with the point spread function, best matched the data. The resulting ring is 1500 ± 100 km wide (3). From the radial profiles we derive an "equivalent width" EW, defined as the radial integral of I/F. For an optically thin ring, EW varies inversely with $\mu \equiv \sin B$; hence, the "normal equivalent width" μEW, equal to what would be measured viewing the rings from above, is better suited for comparisons. Our measurements of R2 from August and October are consistent after allowing for μ, so we adopt their weighted average: $\mu EW = 1.30 \pm 0.13$ m (*3*).

To increase our sensitivity to R1, we flipped the image (Fig. 1B) vertically and then averaged both images. A radial profile was extracted after averaging the data in longitude, but it shows no trace of the ring. Because R1 is quite broad at ~17,000 km (*1*), the limiting factor in its detection is not the intrinsic noise in the data but the systematic uncertainty in the image background. Although this is difficult to quantify, we believe that a ring with $\mu EW = 1$ m might have just marginally escaped detection. We therefore adopt a generous upper limit of $\mu EW = 2.5$ m for R1 (*3*).

Both rings were discovered in HST images through the clear filter (0.3 to 0.7 μm) of the Advanced Camera for Surveys (ACS). Our corresponding measurements from that data set are $\mu EW = 6.46 \pm 0.60$ m for R1 and $\mu EW = 0.63 \pm 0.09$ m for R2. The comparison between the visual and the infrared is striking: R2 is clearly red, whereas R1 is distinctly blue (Fig. 3).

Showalter and Lissauer (*1*) also detected the rings in forward-scattered visual light ("high-phase," phase angle $\alpha = 146°$) in the Voyager images. Using their measurements, we derive the high-phase to low-phase ratio H to be 4.2 ± 0.5 for R1 and 1.6 ± 0.5 for R2. Both rings are predominantly forward-scattering, with $H > 1$. This implies that both rings are very dusty, with particles predominantly in the sub-μm to μm size range. Such tiny grains cannot survive for long in planetary orbit because they are subject to a variety of removal and destruction mechanisms (*4*). Continuous replenishment from larger source bodies is required.

Typical dusty rings, such as Jupiter's ring system and Saturn's G ring, are red. The main rings of Uranus, where particle sizes of ~10 cm

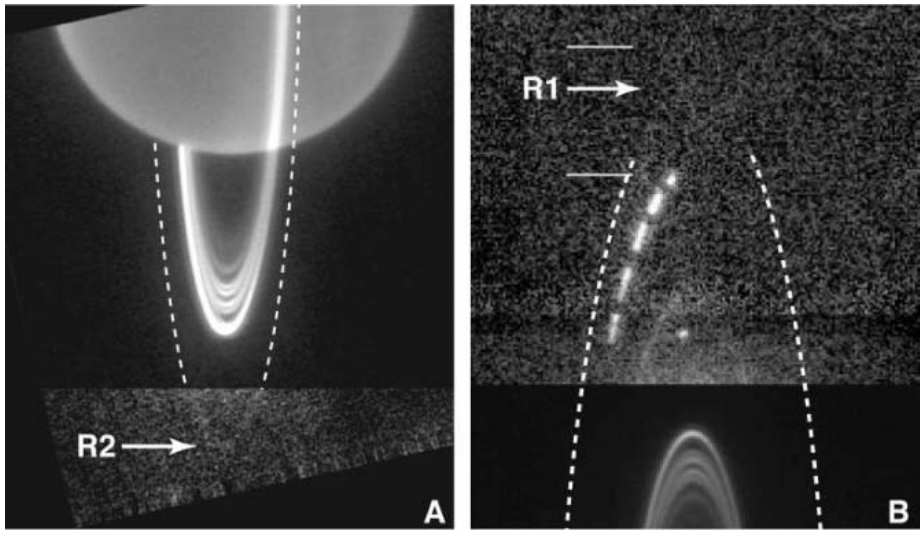

Fig. 1. Keck AO images of the uranian system. Both frames cover ~70,000 km of space vertically, with Uranus south pole pointed to the left. The main rings (their ansas) show prominently. (**A**) R2 is distinctly visible off the southern ansa in August 2005; the bottom portion of this image has been enhanced relative to the planet and main rings to better show the ring. The total integration time was 1 hour. The image has been median-filtered to remove satellites. (**B**) No trace of R1 is visible at the northern ansa in October 2005. This is a coadded mosaic (integration times vary from 48 min to 1.5 hours) in which moving satellites appear as multiple streaks; the most prominent is Puck at 8 to 11 o'clock. The faint streak between Puck and the main rings is Rosalind. Juliet was visible in 1 frame (single dot). Horizontal white lines indicate the full radial limits of R1. R2 is not visible on this image.

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to 10 m dominate (*5*), are essentially neutral in color (*6*, *7*). However, when dust is smaller than or comparable to the wavelength of light, the reflected color is dominated by particle size effects rather than the intrinsic color of the material. For a steep size distribution dominated by the tiniest dust grains, blue light is reflected. For flatter distributions dominated by larger grains, the color is red. Saturn's E ring is the only ring known to be blue (*8*, *9*). Comparing the spectra of rings R1 and R2 with Saturn's distant and dusty E and G rings (Fig. 3), the similarities in spectral slopes between the pairs of rings is striking.

Saturn's G ring and Uranus's R2 show many additional similarities. Both have no known source bodies. Both orbit at similar distances from the planet (Fig. 4): the G ring at 2.78 Saturn radii (R_S) and R2 at 2.64 Uranus radii (R_U). Both have triangular-shaped radial profiles, with a peak displaced inward from the middle of the ring. Both the HST and Keck detections of R2 show evidence for brightness variations with longitude, and Cassini images have revealed arcs in the G ring (*10*). The values in Fig. 3B have not been rescaled; the G and R2 rings are essentially equal in μEW. These rings are also similar in their forward-scattering nature, although uncertainties are large: $H = 2.6 \pm 1.0$ for the G ring in Voyager data (*11*), compared with 1.6 ± 0.5 for R2. However, the available pre-Cassini data are inadequate to constrain the G ring's size distribution uniquely (*12*).

All of these similarities suggest that comparable physical processes are at work in the G and R2 rings. The Pioneer 11 spacecraft detected absorption signatures of high-energy protons while passing over the G ring (*13*). Dust grains cannot absorb such energetic particles; a population of macroscopic bodies, centimeter-sized or larger, must be embedded within the ring (*8*). Collisions between and/or meteoroid impacts into these bodies probably create the G ring. A similar process likely leads to the formation of R2. The variations in longitude may result from gravitational interactions of the dust with larger bodies, either embedded within the ring or acting remotely through resonant interactions.

The blue colors of Saturn's E ring and Uranus's R1 also invite comparison. Perhaps coincidentally, both rings again fall at similar distances from their planets (Fig. 4): 3.95 R_S and 3.82 R_U. Both rings peak in *I/F* near the orbit of a single moon, Enceladus in the case of the E ring and Mab in the case of R1. Both show very broad and again triangular profiles, although the E ring appears to be substantially larger in radial extent. The ring colors are more difficult to compare, because the Keck measurement of R1 is merely an upper limit. Also, the rings are dissimilar in their high-low phase ratios: $H \sim 17$ for the E ring (*8*) but $H \sim 4$ for R1.

Further studies by Cassini at Saturn have not revealed additional moons in the orbit of Enceladus; it appears likely that this moon is the dominant source of the E ring. This is supported by recent images of a plume of dust and gas off the moon's surface (*14*), which may be the dominant source of E-ring material. Mab is smaller in radius by a factor of 20 and is unlikely to be as geologically active as Enceladus; R1's dust is therefore probably generated by the more conventional mechanism of ejecta from meteoroid impacts from Mab.

Given that R1 and R2 probably have similar origins, why are their colors so different? Dynamical simulations of the E ring provide a clue (*4*, *15*, *16*). Although the details vary, studies show that dust grains leaving Enceladus are distributed radially and vertically by a combination of Saturn's oblateness, electromagnetic forces, and solar radiation pressure. The processes act in concert so that particles' paths depend on their sizes. The E ring's blue color arises because tiny particles are distributed most widely, whereas larger grains stay in the vicinity of Enceladus and probably quickly reimpact its surface. Although a detailed study of R1's dynamics is beyond the scope of this paper, it seems plausible that similar processes are at work on dust in the orbit of Mab.

Mab itself was expected to fall within our August and October images, but no pointlike

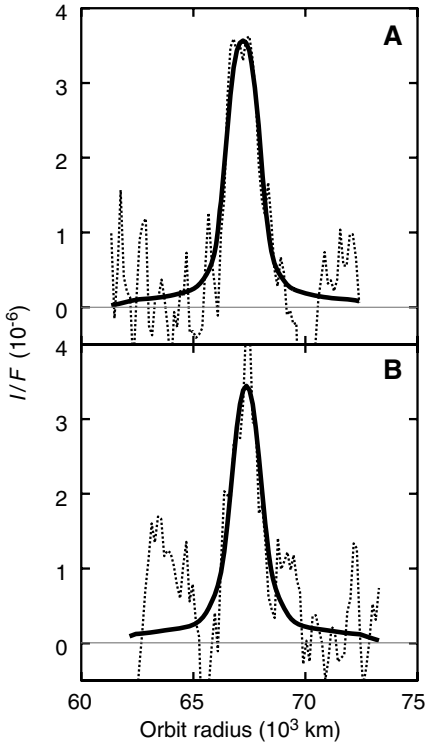

Fig. 2. Radial profiles through the south ansa of ring R2 in August (**A**) and October (**B**). Measured *I/F* is shown as dashed lines; each best fit ring model is overplotted in a heavy solid line. The area under this curve defines the *EW*.

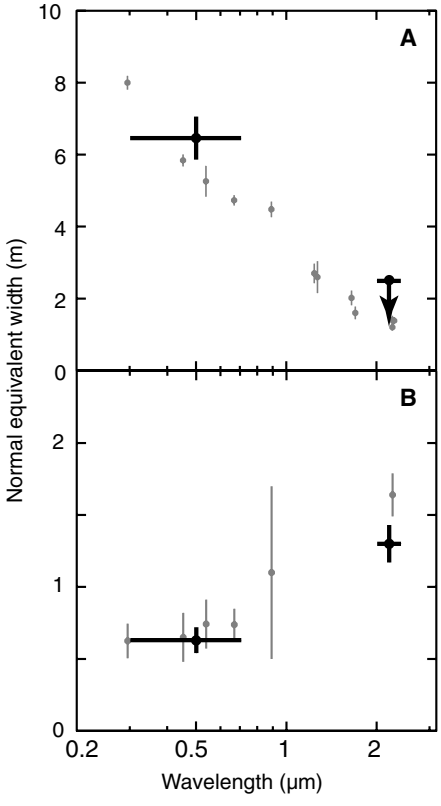

Fig. 3. Spectra of R1 compared with Saturn's E ring (**A**) and R2 compared with the G ring (**B**). Measurements of Uranus' rings are shown in heavy black, Saturn's in gray. Horizontal bars indicate the range of wavelengths averaged; vertical bars indicate $\pm 1\sigma$. The radial integral, μEW, of R1 is compared to an analogous measurement of the E ring (*9*), scaled in intensity to match the R1 values. R2 and the G ring are both radial integrals and are plotted at the same scale.

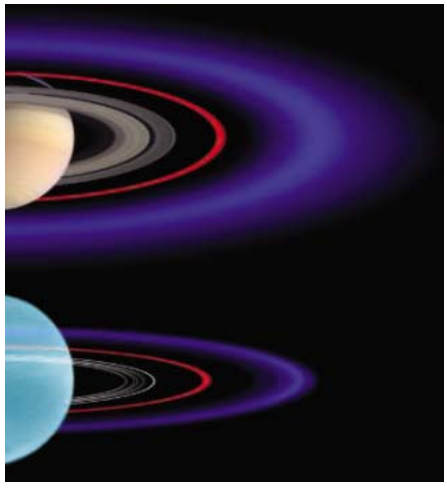

Fig. 4. A schematic view of the outer rings of Saturn and Uranus, in which each system has been scaled to a common planetary radius. We have drawn Uranus' main rings to emphasize that these rings are extremely narrow. In ground-based observations, the rings are blurred (see Fig. 1) because of limited angular resolution (seeing and telescope diameter).

feature was visible near the expected locations. The 3-σ upper limit to the disk-integrated *I/F* of any undetected satellite, derived from individual 120-s exposures, is 30 km^2 (*3*). For comparison, Mab had a disk-integrated *I/F* of 50 km^2 in the HST data, while near maximum elongation as in our October images. This moon is thus very dark near 2 μm, suggestive of absorption by water ice. This makes Mab comparable in surface composition to the five "classical" moons Miranda through Oberon, whose spectra show deep (factor 2 to 3) water ice absorption features near 2 μm. In contrast, Uranus's small inner moons are neutral to slightly red throughout the visual-infrared (*6*, *7*), suggesting that water ice is absent from their surfaces. Mab orbits midway between these two populations. Because of its tiny size, Showalter and Lissauer (*1*) assumed that Mab would resemble the inner moons, with a small geometric albedo (7 to 10%). Our data suggest that Mab may be covered by ice and therefore be as bright as the outer moons (albedo 20 to 40%). This would decrease Mab's estimated radius from 12 km to 6 to 8 km and hence means that Mab, rather than Cupid (~8 km), is Uranus' smallest regular satellite.

In 2007, Uranus' ring system will appear edge-on to Earth, making faint rings ~100 times brighter and enabling measurements of their vertical extent. If indeed similar physical processes are at work within R1 as in Saturn's E ring, then particles in R1 must also be subject to vertical perturbations, resulting in a vertically extended ring. This can be verified in 2007.

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Supporting Online Material

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Materials and Methods
References

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Deconvolution of the Factors Contributing to the Increase in Global Hurricane Intensity

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To better understand the change in global hurricane intensity since 1970, we examined the joint distribution of hurricane intensity with variables identified in the literature as contributing to the intensification of hurricanes. We used a methodology based on information theory, isolating the trend from the shorter-term natural modes of variability. The results show that the trend of increasing numbers of category 4 and 5 hurricanes for the period 1970–2004 is directly linked to the trend in sea-surface temperature; other aspects of the tropical environment, although they influence shorter-term variations in hurricane intensity, do not contribute substantially to the observed global trend.

Recent publications linking an increase in hurricane intensity to increasing tropical sea-surface temperatures (SSTs) (*1*–*5*) have fueled the debate on whether global warming is causing an increase in hurricane intensity (*6*, *7*). The arguments associating the increase in hurricane intensity with increasing SSTs (*1*) note positive trends in both global tropical SST and the number of category 4 and 5 hurricanes (NCAT45). The physical mechanism linking the increases in tropical SST and NCAT45 is the theory of maximum potential intensity (*3*).

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The analysis presented in (*1*) established the existence of coincident positive trends of tropical SST and NCAT45 in each of the ocean basins. Outstanding issues in understanding the substantial increase in global NCAT45 since 1970 include (i) identification of the contributions of natural internal variability on decadal and shorter time scales as compared to a longer-term trend and (ii) identification of the importance of SST in causing the increase in NCAT45, relative to other known variables that influence hurricane intensity (*8*, *9*).

To address these issues, we analyzed the time series of SST, specific humidity in the layer extending from 925 to 500 millibars (mb), wind shear between 850 and 200 mb, and the 850-mb zonal stretching deformation (the change of zonal wind with longitude). Increasing SST, increasing specific humidity, minimal vertical wind shear, and negative stretching deformation are associated with increasing hurricane intensity. The data sets used in this analysis were the hurricane data set described in (*1*), the National Oceanic and Atmospheric Administration Extended Reconstructed SST data set (*10*), and the National Centers for Environmental Prediction/National Center for Atmospheric Research Reanalysis (*11*). The analysis was conducted for seasonally averaged values for the period 1970–2004 for global hurricanes, including data from the North Atlantic (NATL), West Pacific (WPAC), East Pacific (EPAC), South Pacific (SPAC), South Indian (SIO), and North Indian (NIO) Oceans. Hence, the data set consisted of data points for 35 seasons and six different ocean basins, for a total sample size of 210.

Figure 1 shows a clear positive trend in SST in each of the ocean basins. Although there is no consistent global trend of specific humidity, wind shear, and stretching deformation, there are statistically significant trends [as per the Mann-Kendall approach (*12*)] in several of the basins (Table 1): in the EPAC, a positive trend in specific humidity; in the SPAC, a negative trend in stretching deformation at 850 mb; and in the NATL, a negative trend in wind shear. It is important to note that these particular trends reinforce the necessary conditions for hurricane formation. The SST trends are not only statistically significant in each of the ocean basins but also have the largest magnitude, except for the SPAC, where stretching defor-

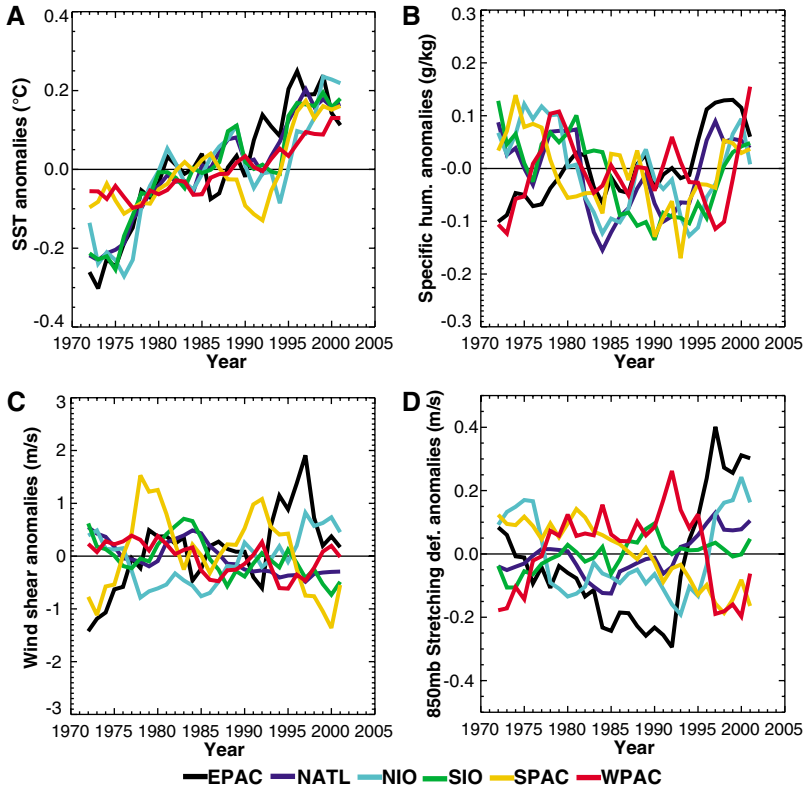

Fig. 1. Five-year moving average anomalies relative to the 1970–2004 period for the EPAC (90° to 120°W, 5° to 20°N, June-October), NATL (90°W to 20°E, 5° to 25°N, June-October), NIO (55° to 90°E, 5° to 20°N, April-May and September-November), SIO (50° to 115°E, 5° to 20°S, November-April), SPAC (155° to 180°E, 5° to 20°S, December-April), and WPAC (120° to 180°E, 5°to 20°N, May-December). Basins and seasons are defined by (*1*) for (**A**) SST, (**B**) specific humidity, (**C**) wind shear, and (**D**) stretching deformation at 850 mb.

Table 1. Standardized trends (per 35 years) for all variables in each basin for the period 1970–2004.

Basin	SST	Specific humidity	Wind shear	Stretching deformation (850 mb)
EPAC	1.934*	1.228*	0.827	0.581
NATL	2.767*	0.058	−1.880*	1.208
NIO	2.017*	−0.971	0.315	−0.134
SIO	2.095*	−0.653	−1.468	0.734
SPAC	1.788*	−0.159	−0.035	−1.840*
WPAC	2.046*	0.803	−0.562	0.376

*Values are significantly different than zero at the 99% confidence level.

mation at 850 mb also presents a trend of comparable magnitude.

To understand the relationships between NCAT45 and the four variables considered, we used a methodology based on information theory, whereby the mutual information (MI) of both variables is quantified to represent the measure of independence of the two variables (*13*). Specifically, the MI quantifies the distance between the joint distribution of two variables, X and Y, and the product of their marginal distributions. MI can be thought of as a measure of the information of X that is shared by Y. If X and Y are independent, then X contains no information about Y and vice versa, so their MI is zero [see supporting online material (*14*)].

To quantify the MI, it is necessary to estimate the marginal distribution of the variables. Figure 2, A and B, shows the distributions of NCAT45 and SST. If these two variables were statistically independent, the product of their marginal distributions should replicate their joint distribution (Fig. 2C). The fact that this is not seen for NCAT45 and SST implies that there is statistical dependence between the variables. The joint distribution (Fig. 2D) indicates that statistically, the average SST during each basin's hurricane season should be high in order to have a larger value of NCAT45. That is, high SST is important not only during the lifetime of a single event (*15*, *16*), but the average SST conditions of the basin seem to be highly linked with the probability of occurrence of NCAT45. The scaled distribution [the joint distribution scaled by the marginal distribution (Fig. 2E)] shows that higher values associated with their MI [0.51 bits (*17*)] are located along the diagonal and mainly in its upper part, confirming that the seasonal values of SST are intimately related to NCAT45.

To address the issue of the relative importance of the MI between NCAT45 and SST versus the other variables, we performed the same MI statistical analysis. For specific humidity (Fig. 3A), the analysis indicates that the MI is 0.49 bits. Higher seasonal average values of specific humidity are associated with higher values of NCAT45, with no high NCAT45 associated with low values of specific humidity. Wind shear (Fig. 3B) also shows a relationship with NCAT45 (0.44 bits), with values associated with high values of NCAT45 in the bottom half of the distribution. In other words, high NCAT45 is more likely to occur when the wind shear tends to be low relative to its distribution. In the case of stretching deformation (Fig. 3C), it is clear that the occurrence of high NCAT45 always corresponds to negative values of stretching deformation (*9*) and explains most of the MI, which is 0.68 bits.

The values of the MI presented in Figs. 2 and 3 are roughly comparable in magnitude, ranging from a high value of 0.68 bits for stretching deformation to a low value of 0.44 bits for wind shear. Because this analysis does not directly allow for distinguishing whether these relationships arise from the long-term trend or the shorter-term modes of natural internal variability (on a decadal scale or shorter), we performed the MI analysis on the isolated trend/variability time series (*14*). The trend is removed by subtracting the least-squares linear fit from each basin's variable time series. This method has a physical interpretation only if the trend has a consistent sign in all of the ocean basins and is statistically significant. Hence, this particular analysis is applied only to SST.

Figure 4 shows the results for SST trend time series (Fig. 4A) and SST variability time series (Fig. 4B). The MI is higher when the trend (0.54 bits), in contrast to the variability (0.30 bits), is isolated, and the trend value is comparable to that of the original signal (0.51 bits). In addition, it can be observed that the scaled distribution of the trend is very similar to that of the original variable (Fig. 4A versus Fig. 2E). The high values of NCAT45 appear to be associated with high SST (the upper part of the diagonal) when the trend is isolated (Fig. 4A); however, this is not the case for the variability, which is very symmetrical around the median (Fig. 4B). This implies statistically that the trend in SST accounts for the information associated with the occurrence of high values of NCAT45, whereas the shorter-term variability in SST does not account for a

large proportion of the variance of the high NCAT45.

Recently, the quality of the hurricane data has been questioned (*4*, *5*), and a reanalysis of the tropical cyclone databases has even been suggested in order to confirm that the results of recent studies (*1*, *2*) are not due to problems in the data. Because the NIO hurricane data set has been directly questioned because of potential problems, especially during the 1970s, we performed the MI analysis excluding this basin, obtaining similar results pointing to the same conclusions reached when all six basins were used (fig. S4). In addition, we analyzed the behavior of the seasonal tropospheric moist static stability for all six basins. We found a statistically significant and strong negative trend in this variable for five of the six basins (fig. S5), indicating a more unstable troposphere. Interestingly, although the EPAC shows no trend in the moist static stability index, it is the only basin that presents a significant positive trend in the specific humidity (Table 1). These results support the physical connection between ocean and atmosphere for the link between increasing SST and NCAT45.

An outstanding issue is to investigate whether the SST variation is the primary source of information shared with the NCAT45 trend in the three basins that possessed statistically significant trends in other variables: the EPAC specific humidity, the SPAC stretching deformation at 850 mb, and the NATL vertical wind shear. Because the number of data points for each basin is only 35, we could not apply the MI technique. Hence, we conducted a correlation analysis on the complete and detrended time series for each of these basins (Table 2). The correlation between humidity and NCAT45 in the EPAC is not statistically significant. The correlation between stretching deformation and NCAT45 in the SPAC is statistically significant, but the correlation arises almost entirely from the short-term internal variability. The correlation between wind shear and NCAT45 in the NATL is statistically significant, but the correlation is dominated by the short-term variability.

Unfortunately, the sample size for the individual basins is too small to reliably test the significance of the difference between the correlation for the original time series and that for the detrended time series. The same correlation analysis shown in Table 2 for SST (*18*) reveals that the correlation for SST in the original time series is more than twice as large as for the detrended time series. This analysis suggests that the only basins where there may be some significant contribution of trend from variables besides SST are the NATL and the SPAC. Although this analysis cannot determine quantitatively the relative contributions of SST versus vertical wind shear to the NCAT45 trend in the NATL and versus zonal stretching deformation in the SPAC, the relative differences in the magnitudes of correlations for the complete versus the detrended time series indicate that the contribution from SST dominates in both basins in contributing to the trend in NCAT45.

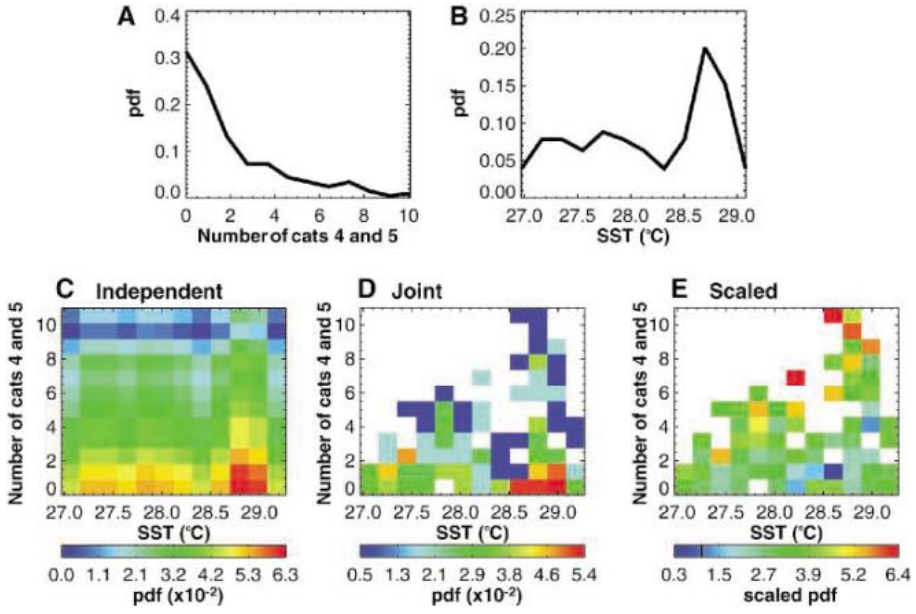

Fig. 2. Marginal distributions in all basins for (**A**) NCAT45 and (**B**) SST. pdf, probability density function. (**C**) Product of the marginal distributions, (**D**) joint distribution, and (**E**) scaled distribution for (A) and (B).

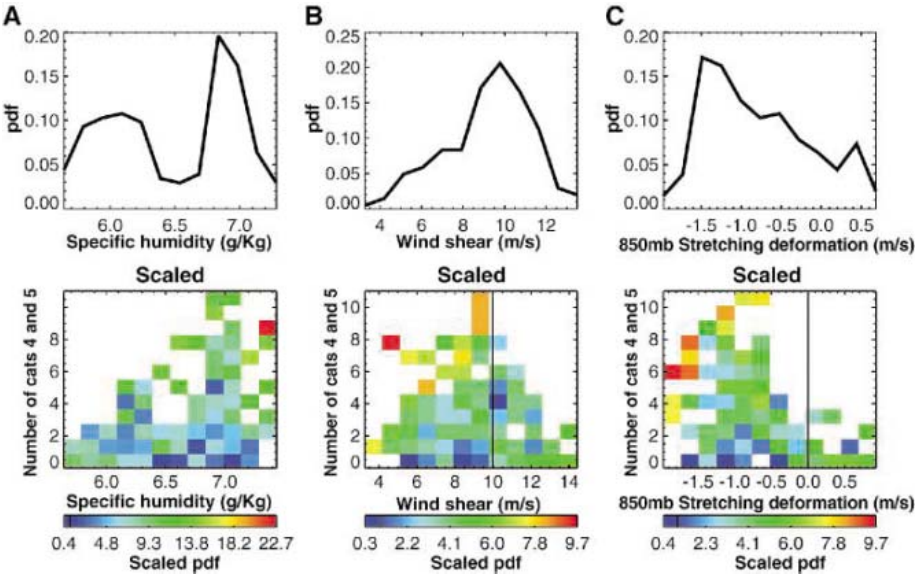

Fig. 3. (**A** to **C**) Marginal and scaled distribution for (B) to (D) in Fig. 1. The vertical black line in the bottom panels of (B) and (C) corresponds to the median of the distribution in (B) and to zero in (C).

Our results show that seasonally averaged values of tropical SST, tropospheric humidity, vertical wind shear, and zonal stretching deformation share information content with the total number of NCAT45 in a season. The shared information content with tropospheric humidity, vertical wind shear, and zonal stretching deformation is dominated by short-term variability, whereas the shared information content with SST is dominated by the longer-term trend. The implication of these results is that the strong increasing trend in NCAT45 for the period 1970–2004 is directly linked to the trend in tropical SST, and that other aspects of the tropical environment, although they influence shorter-term variations in hurricane intensity, do not contribute significantly to the global trend of increasing hurricane intensity. We infer that there is some contribution to the long-term trend from wind shear in the NATL and from stretching deformation in the SPAC, but that the contribution from SST remains dominant in these basins in contributing to the trend in NCAT45.

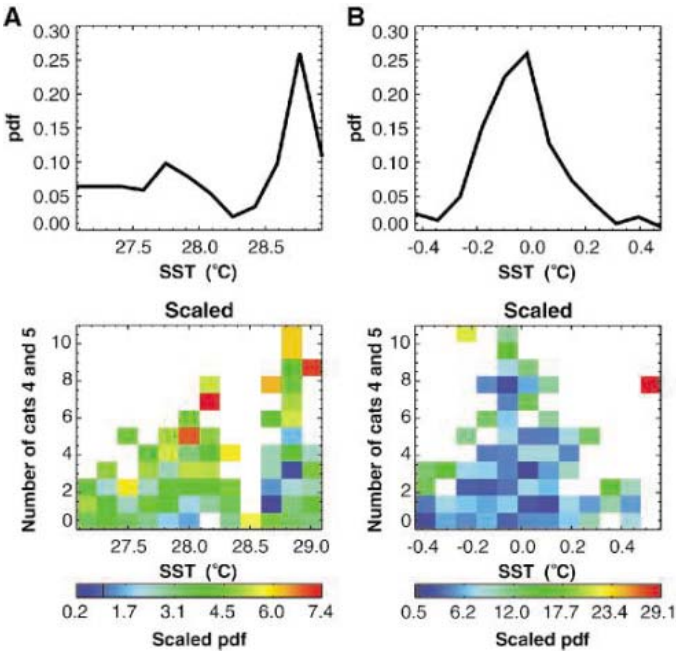

Fig. 4. Marginal and scaled distribution for (**A**) the SST trend time series and (**B**) the detrended SST variability time series. Values greater than one (the vertical line in the color bar at the bottom) account for the MI shared between the variables.

Table 2. Correlations for individual ocean basins between NCAT45 and variables for which there is a statistically significant trend in that basin, for the original time series in bold and the detrended time series in parentheses. NA, not applicable.

Variable	EPAC	NATL	SPAC
SST	**0.32*** (0.06)	**0.67*** (0.20)	**0.29** (–0.10)
Specific humidity	**0.23** (0.13)	NA	NA
Wind shear	NA	**–0.61*** (–0.45)*	NA
Stretching deformation	NA	NA	**–0.67*** (–0.55)*

*Statistically significant correlations at the 95% confidence level.

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17. Values of MI vary from 0 (total independence) to 2.8 (total dependence), corresponding to the entropy of NCAT45 (minimum among all variables).
18. SST correlations for the remaining three basins are as follows (using the same notation as in Table 2): WPAC **0.44*** (0.13), NIO **0.28** (–0.10), SIO **0.54*** (0.02).

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Evolution of Hormone-Receptor Complexity by Molecular Exploitation

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According to Darwinian theory, complexity evolves by a stepwise process of elaboration and optimization under natural selection. Biological systems composed of tightly integrated parts seem to challenge this view, because it is not obvious how any element's function can be selected for unless the partners with which it interacts are already present. Here we demonstrate how an integrated molecular system—the specific functional interaction between the steroid hormone aldosterone and its partner the mineralocorticoid receptor—evolved by a stepwise Darwinian process. Using ancestral gene resurrection, we show that, long before the hormone evolved, the receptor's affinity for aldosterone was present as a structural by-product of its partnership with chemically similar, more ancient ligands. Introducing two amino acid changes into the ancestral sequence recapitulates the evolution of present-day receptor specificity. Our results indicate that tight interactions can evolve by molecular exploitation—recruitment of an older molecule, previously constrained for a different role, into a new functional complex.

The ability of mutation, selection, and drift to generate elaborate, well-adapted phenotypes has been demonstrated theoretically (*1*, *2*), by computer simulation (*3*, *4*), in the laboratory (*5*, *6*), and in the field (*7*). How evolutionary processes assemble complex systems that depend on specific interactions among the parts is less clear, however. Simultaneous emergence of more than one element by mutational processes is unlikely, so it is not apparent how selection can drive the evolution of any part or the system as a whole. Most molecular processes are regulated by specific interactions, so the lack of exemplars for the emergence of such systems represents an important gap in evolutionary knowledge. As Darwin stated, "If it could be demonstrated that any complex organ existed which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down" (*8*).

The functional interaction between the steroid hormone aldosterone and its specific partner the mineralocorticoid receptor (MR)—a ligand-activated transcriptional regulator (*9*, *10*)—illustrates this evolutionary puzzle. MR and the glucocorticoid receptor (GR) descend from a gene duplication deep in the vertebrate lineage (*11*) and now have distinct signaling functions. In most vertebrates, GR is specifically activated by the stress hormone cortisol to regulate metabolism, inflammation, and immunity (*9*). MR is activated by aldosterone to control electrolyte homeostasis and other processes (*9*, *12*). MR can also be activated by cortisol, although the presence of a cortisol-

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clearing enzyme in many MR-expressing tissues makes the receptor a largely aldosterone-specific factor (*12*). It is not obvious how the tight aldosterone-MR partnership could have evolved. If the hormone is not yet present, how can selection drive the receptor's affinity for it? Conversely, without the receptor, what selection pressure could guide the evolution of the ligand?

To reconstruct the evolution of the MR's interaction with aldosterone, we characterized the functions of the ancestral corticoid receptor (AncCR)—the ancient protein from which GR and MR descend by gene duplication. To improve the robustness of this inference, we first isolated corticoid receptor sequences from basal vertebrate taxa. Using the polymerase chain reaction (*13*), we identified a single corticoid receptor in jawless fishes—the lamprey *Petromyzon marinus* and the hagfish *Myxine glutinosa*—and both GR and MR in an elasmobranch, the skate *Raja erinacea*. Phylogenetic analysis indicates that the duplication leading to GR and MR occurred >450 million years ago, after the divergence of jawless fishes but before the split of cartilaginous fish from bony vertebrates (Fig. 1 and supplementary figs. S1 to S3). Functional assays (*13*) indicate that the basal receptors are activated by very low doses of aldosterone, cortisol, and 11-deoxycorticosterone (DOC); they are similar in this respect to MRs of tetrapods and teleosts (Fig. 2 and figs. S4 and S5) (*14*–*16*). The only receptors insensitive to aldosterone are the GRs of tetrapods and teleosts.

Given these results, the most parsimonious scenario is that AncCR was capable of being activated by aldosterone and that aldosterone sensitivity was lost in the GRs of bony vertebrates (Fig. 1). To test this hypothesis, we used gene resurrection (*17*) to experimentally characterize the ancestral CR. On the basis of the ML phylogeny and a large alignment of extant receptor sequences (table S1), we inferred the maximum likelihood (ML) amino acid sequence of AncCR's ligand-binding domain (LBD), the functionally separable region that contains the protein's ligand-regulated transcriptional functions (*13*). The ancestral sequence was inferred with strong support: The mean posterior probability (PP) was 94%, and two-thirds of sites had PP > 99% (table S2). AncCR-LBD is most similar to aldosterone-activated receptors MRs and CRs and differs from them by just one residue in the ligand-binding pocket (table S3).

We synthesized the AncCR-LBD sequence and expressed it in cultured cells; using a reporter assay, we found that AncCR is a sensitive and effective aldosterone receptor (*13*). Like the extant CRs and MRs, it is also activated by low doses of DOC and, to a lesser extent, cortisol (Fig. 3A). AncCR's aldosterone sensitivity is robust to uncertainty about the phylogeny and stochastic error in the sequence reconstruction. We used Bayesian phylogenetics to collect a large sample of plausible trees and reconstructed AncCR-LBD on all 467 trees in the 95% credible set; the ancestral sequence on every tree was identical to that on the ML tree, except for one site. When the alternate state was introduced into the reconstructed protein, AncCR became even more sensitive to aldosterone (fig. S6). To characterize AncCR's robustness to stochastic error, we examined positions that had an alternate state with PP > 0.20. In all cases but one, the alternate state is found in other aldosterone-activated receptors and is therefore not sufficient to abolish aldosterone sensitivity; introducing the exception into AncCR had no effect on ligand-activation (fig. S6). Finally, among sites that make contact with the ligand in the MR crystal structure (*18*), only one was ambiguously reconstructed. The mutagenized AncCR with the alternate state remained highly sensitive to aldosterone (fig. S6).

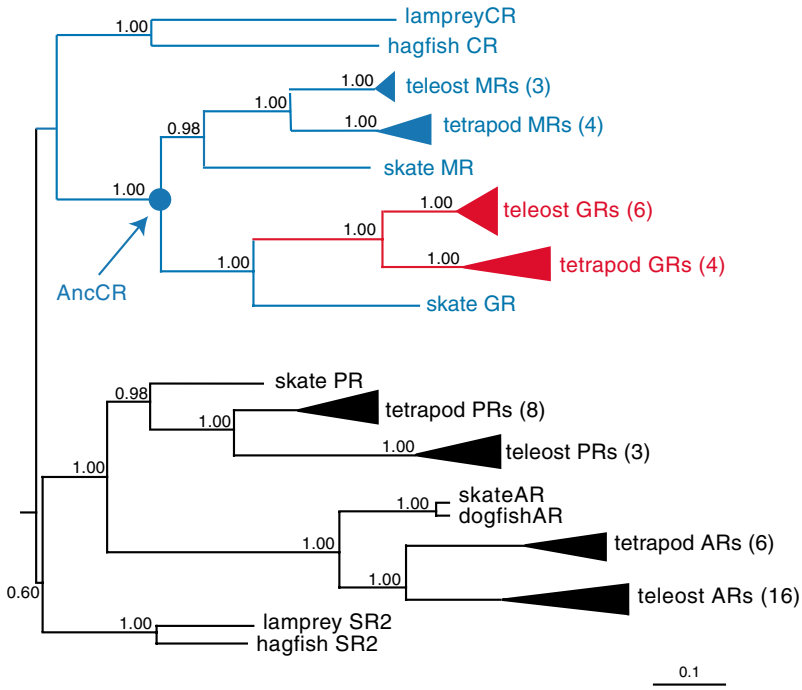

Fig. 1. Phylogeny of steroid hormone receptors. The gene family tree of 59 steroid and related receptor amino acid sequences was inferred using maximum likelihood (ML), Bayesian Markov chain Monte Carlo (BMCMC), and maximum parsimony (*13*). ML branch lengths and BMCMC posterior probabilities for major nodes are shown. The number of sequences in each clade is in parentheses. The ancestral corticoid receptor (AncCR) is marked with a circle. Blue, aldosterone-activated receptors; red, aldosterone-insensitive glucocorticoid receptors; black, noncorticoid receptor outgroups. For complete phylogenies and sequences, see figs. S1 to S3 and table S1.

The aldosterone activation of AncCR—like that of the agnathan, elasmobranch, and teleost receptors—is surprising, because aldosterone has long been considered a tetrapod-specific hormone. Using a very sensitive fractionation and immunodetection strategy (*13*), we confirmed that aldosterone is absent from the plasma of lamprey and hagfish (Fig. 3B). Further, when interrenal gland explants were incubated with appropriate precursors and stimulatory hormones, neither species produced aldosterone (fig. S7). Aldosterone has been reliably detected in tetrapods (*9*), but is absent from teleosts (*19*), elasmobranchs (*20*, *21*), and agnathans, as our experiments show. The capacity to synthesize aldosterone therefore evolved relatively recently, in the lineage leading to tetrapods. Aldosterone's emergence was due to modification of cytochrome P-450 11β-hydroxylase, the ancestral function of which is to hydroxylate DOC in glucocorticoid synthesis, a function present in all jawed vertebrates. Only in tetrapods has this enzyme evolved the additional capacity to hydroxylate corticosterone, allowing aldosterone synthesis (Fig. 4A) (*19*, *22*, *23*).

The sensitivity of corticoid receptors to aldosterone is therefore more ancient than the hormone itself (Fig. 4B). AncCR must have been regulated by a different ligand; one candidate is DOC, which is produced by agnathans (*24*) and by all jawed vertebrates as an intermediate in the synthesis of other corticosteroids (Fig. 4A). AncCR and the agnathan CRs, like the MRs of tetrapods and teleosts (*15*, *16*), are very sensitive to DOC (Fig. 3A and fig. S4). Whatever the precise identity of the ancestral ligand, AncCR's aldosterone responsiveness, like that of CRs and MRs in species that do not produce the hormone, is due to aldosterone's structural similarity to steroids that do activate the receptor. Aldosterone differs from DOC only by small moieties at the 18- and 11-positions; our experiments show that neither of these affects activation of the ancestral or extant CRs.

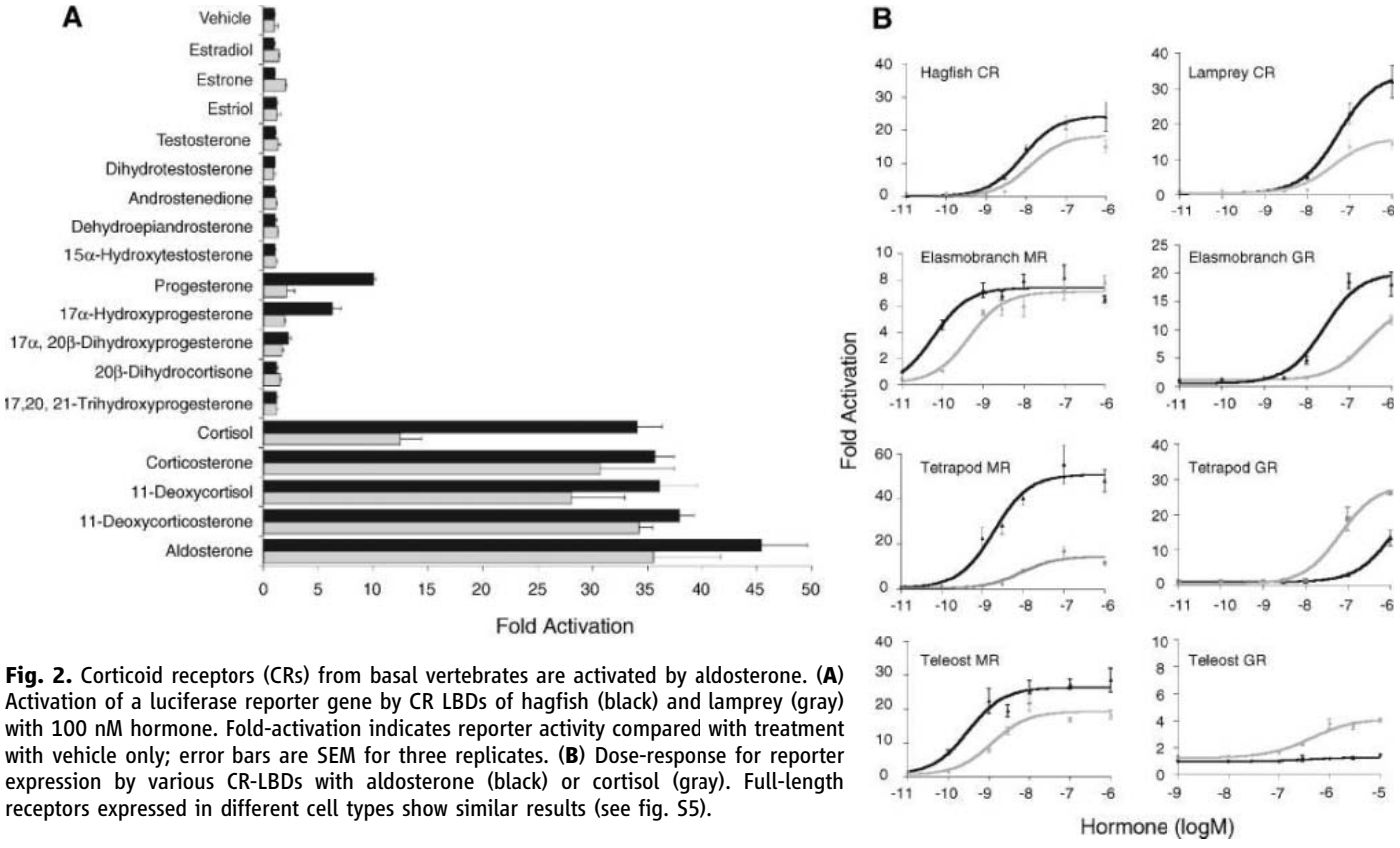

Fig. 2. Corticoid receptors (CRs) from basal vertebrates are activated by aldosterone. (**A**) Activation of a luciferase reporter gene by CR LBDs of hagfish (black) and lamprey (gray) with 100 nM hormone. Fold-activation indicates reporter activity compared with treatment with vehicle only; error bars are SEM for three replicates. (**B**) Dose-response for reporter expression by various CR-LBDs with aldosterone (black) or cortisol (gray). Full-length receptors expressed in different cell types show similar results (see fig. S5).

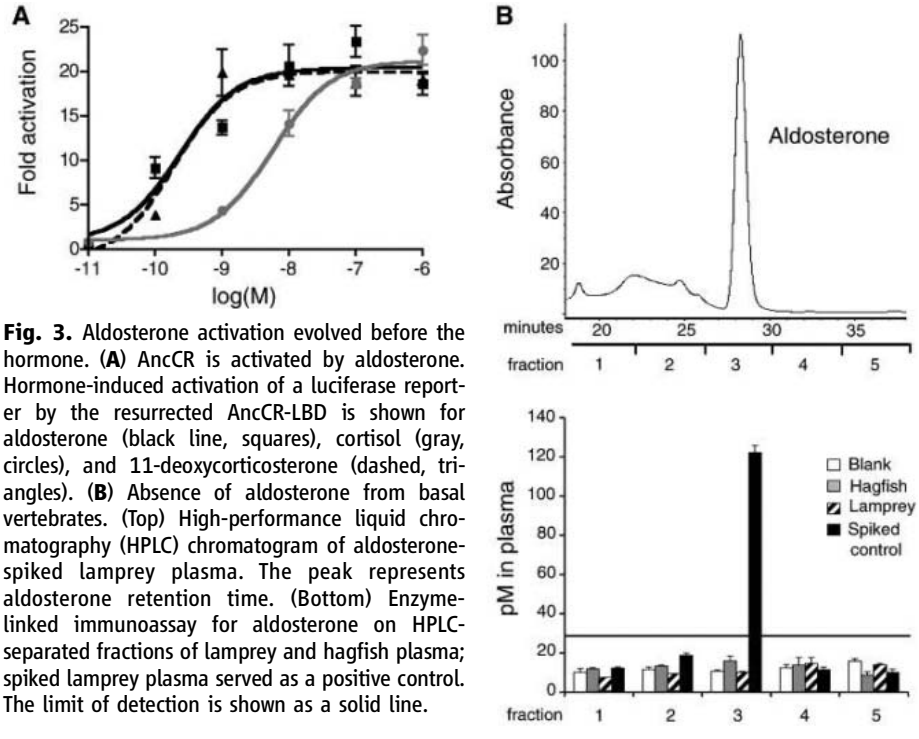

Fig. 3. Aldosterone activation evolved before the hormone. (**A**) AncCR is activated by aldosterone. Hormone-induced activation of a luciferase reporter by the resurrected AncCR-LBD is shown for aldosterone (black line, squares), cortisol (gray, circles), and 11-deoxycorticosterone (dashed, triangles). (**B**) Absence of aldosterone from basal vertebrates. (Top) High-performance liquid chromatography (HPLC) chromatogram of aldosterone-spiked lamprey plasma. The peak represents aldosterone retention time. (Bottom) Enzyme-linked immunoassay for aldosterone on HPLC-separated fractions of lamprey and hagfish plasma; spiked lamprey plasma served as a positive control. The limit of detection is shown as a solid line.

Extant MRs retain the ancestral phenotype, so the specificity of the MR-aldosterone relationship is due to the secondary loss of aldosterone sensitivity in the GR (Fig. 4B). To understand the mechanistic basis for this functional shift, we identified sequence changes that are phylogenetically and functionally diagnostic, defined as having occurred on the branch where aldosterone sensitivity was lost, with one state conserved in all the aldosterone-activated receptors and a different state in all aldosterone-insensitive GRs. We introduced all four single GR-diagnostic states and all six twofold combinations into AncCR-LBD using mutagenesis and determined their effect on receptor function. One combination—replacement of Ser^{106} with Pro (S106P) and Leu^{111} with Gln (L111Q) (numbered by position in AncCR-LBD)—conferred a GR-like phenotype: The receptor's median effective concentration (EC_{50}) for aldosterone increased by three orders of magnitude, but moderate cortisol and DOC sensitivity were retained (Fig. 4C). None of the other mutants showed this pattern (table S4). Structural studies of the human GR have shown that these two residues change the architecture of the ligand-binding pocket and alter contacts with steroid in ways that exclude aldosterone and facilitate cortisol-activation (*18*, *25*). Our data thus indicate that the aldosterone specificity of MR has a simple and conserved mechanistic basis—two crucial replacements in the GRs that wiped out ancestral sensitivity to aldosterone.

To reconstruct the trajectory of GR sequence evolution, we introduced each replacement in isolation and found that both are required to yield the GR phenotype. L111Q alone radically reduces activation by all ligands tested (Fig. 4C). In contrast, S106P reduces aldosterone and cortisol sensitivity, but this receptor remains highly DOC-sensitive. In the S106P background, L111Q further reduces aldosterone sensitivity but now restores cortisol response to levels character-

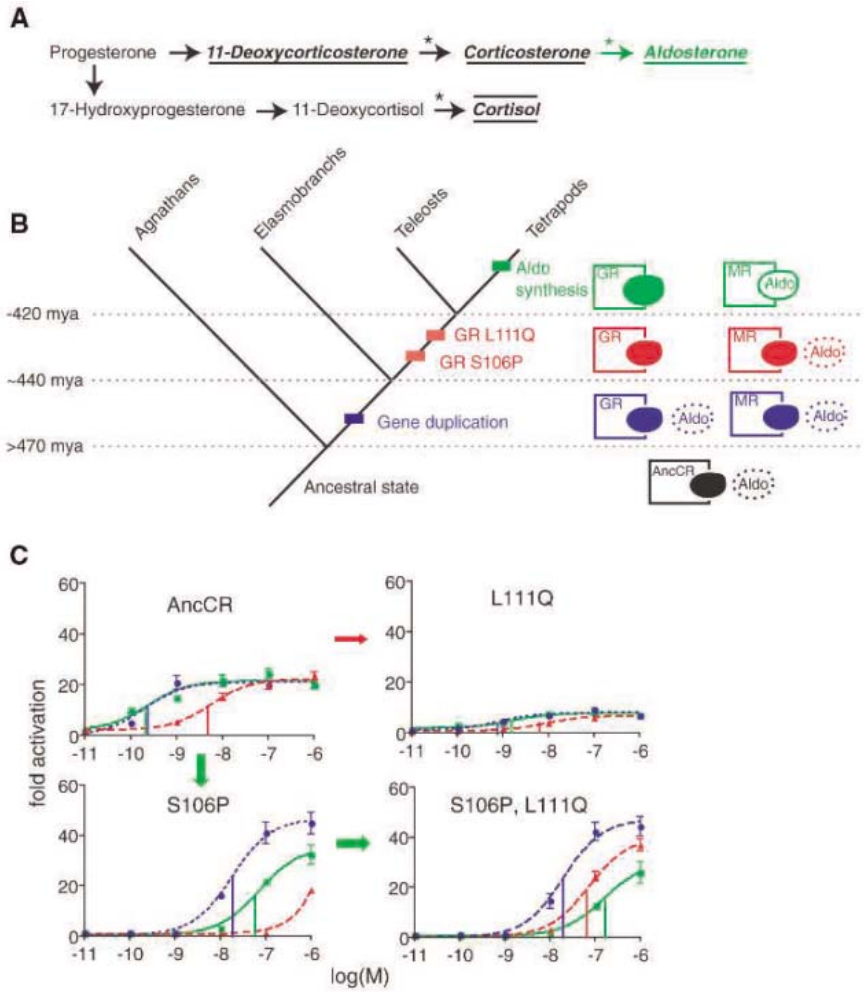

Fig. 4. Evolution of specific aldosterone-MR signaling by molecular exploitation. (**A**) Synthesis pathway for corticosteroid hormones. Ligands for the ancestral CR and extant MRs are underlined; cortisol, the ligand for the tetrapod GR, is overlined. The terminal addition of aldosterone is in green. Asterisks, steps catalyzed by the cytochrome P-450 11β-hydroxylase enzyme; only the tetrapod enzyme can catalyze the step marked with a green asterisk. (**B**) MR's aldosterone sensitivity preceded the emergence of the hormone. The vertebrate ancestor did not synthesize aldosterone (dotted circle), but it did produce other corticosteroids (filled circle); it had a single receptor with affinity for both classes of ligand. A gene duplication (blue) produced separate GR and MR. Two changes in GR's sequence (red) abolished aldosterone activation but maintained cortisol sensitivity [see (C)]. In tetrapods, synthesis of aldosterone emerged due to modification of cytochrome P-450 11β-hydroxylase. mya, million years ago. (**C**) Mechanistic basis for loss of aldosterone sensitivity in the GRs. Phylogenetically diagnostic amino acid changes that occurred during GR evolution were introduced into AncCR-LBD by mutagenesis. Dose-response is shown for aldosterone (green), DOC (blue), and cortisol (red). The double mutant (bottom right) has a GR-like phenotype. Arrows shows evolutionary paths via a nonfunctional (red) or functional (green) intermediate.

istic of extant GRs. A mutational path beginning with S106P followed by L111Q thus converts the ancestor to the modern GR phenotype by functional intermediate steps and is the most likely evolutionary scenario (*26*).

Our findings demonstrate that the MR-aldosterone partnership evolved in a stepwise fashion consistent with Darwinian theory, but the functions being selected for changed over time. AncCR's sensitivity to aldosterone was present before the hormone, a by-product of selective constraints on the receptor for activation by its native ligand. AncCR and its descendant genes were structurally preadapted for activation by aldosterone when that hormone evolved millions of years later. After the duplication that produced GR and MR, only two substitutions in the GR lineage were required to yield two receptors with distinct hormone-response profiles. The evolution of an MR that could be independently regulated by aldosterone enabled a more specific endocrine response, because it allowed electrolyte homeostasis to be controlled without also triggering the GR stress response, and vice versa.

This evolutionary scenario—recruiting an ancient receptor into partnership with a novel ligand—is the obverse of the dynamic previously established for the androgen and progestin receptors (AR, PR). In that case, duplicates of an ancient estrogen-responsive receptor evolved affinity for steroids that previously served as intermediates in estrogen synthesis (*11*, *27*). Together, the hormone-first history of AR and PR and the receptor-first history of MR point to a general evolutionary dynamic: Novel interactions emerge when a newly generated molecule—usually a slight structural modification or duplicate of an existing one—recruits as a binding partner a more ancient molecule, which was previously constrained by selection for an entirely different function. This model, which we call "molecular exploitation," is consistent with findings that other ancient biological features have been co-opted for novel functions (*28*–*30*).

The puzzle that complex systems pose for Darwinian evolution depends on the premise that each part has no function—and therefore cannot be selected for—until the entire system is present. This puzzle might indeed cause Darwin's theory to "break down" if the functions of the parts must remain static for all time. But virtually all molecules can and do participate in more than one process or interaction, so a complex's elements may have been selected in the past for unrelated functions. Our work indicates that tightly integrated systems can be assembled by combining old molecules with different ancestral roles together with new ones—generated by gene duplication or elaboration of enzymatic pathways—that represent slight structural variants on older elements. We propose that molecular exploitation will be a predominant theme in evolution, one that may provide a general explanation for how the molecular interactions critical for life's complexity emerged in Darwinian fashion.

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Phylogeny of the Ants: Diversification in the Age of Angiosperms

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We present a large-scale molecular phylogeny of the ants (Hymenoptera: Formicidae), based on 4.5 kilobases of sequence data from six gene regions extracted from 139 of the 288 described extant genera, representing 19 of the 20 subfamilies. All but two subfamilies are recovered as monophyletic. Divergence time estimates calibrated by minimum age constraints from 43 fossils indicate that most of the subfamilies representing extant ants arose much earlier than previously proposed but only began to diversify during the Late Cretaceous to Early Eocene. This period also witnessed the rise of angiosperms and most herbivorous insects.

Ants are a ubiquitous and dominant feature of the terrestrial landscape, playing key roles in symbiotic interactions, soil aeration, and nutrient cycling. They have a rich fossil record (*1*), yet the evolutionary history of the ~11,800 described modern species remains poorly resolved.

Bolton's (*1*) recent revision of ants (Hymenoptera: Formicidae) recognized 288 genera in 21 [subsequently reduced to 20 (*2*, *3*)] subfamilies. Several phylogenies have been proposed based primarily on morphological characters, but these reflect disagreement about the positions of major lineages (fig. S2) (*4*–*7*). Recent molecular analyses have included only a moderate number of taxa and recovered only weak support for most clades (*8*, *9*), although more comprehensive studies are under way (*10*).

To evaluate competing phylogenetic hypotheses, we analyzed 4.5 kb of sequence data (Fig. 1) from portions of five nuclear genes and one mitochondrial gene from 139 ant genera and six Aculeatea Hymenoptera outgroups (n = 149 specimens) representing 19 of the 20 currently recognized extant subfamilies. The only ant subfamily not included was Aenictogitoninae, a rare group known only from males collected at lights in equatorial Africa. The monophyly of the Formicidae itself was strongly supported in all analyses (Table 1).

Analyses with several methods (*11*) resulted in a well-resolved phylogeny that divided the family into three groups: the leptanilloid clade, a basal lineage containing 1 subfamily (Leptanillinae) and sister to all other ants; the poneroid clade, containing 5 subfamilies (Agroecomyrmecinae, Amblyoponinae, Paraponerinae, Ponerinae, and Proceratiinae); and the formicoid clade, containing the remaining 13 subfamilies sampled in this study. All three clades were supported by 100% Bayesian posterior probability (bpp) support, but only the formicoid and leptanilloid clades were well supported in the maximum likelihood analyses [≥94% maximum likelihood bootstrap (ml bs)].

Of the 19 subfamilies investigated here, 14 were recovered as monophyletic with strong support, and none of the three monotypic taxa, each represented by a single extant species (Agroecomyrmecinae, Aneuretinae, and Paraponerinae), nested within another lineage, validating their status as separate subfamilies. However, the three sampled genera of Cerapachyinae were paraphyletic in all analyses. The eight genera of Amblyoponinae grouped together in a clade that lacked support, although the monophyly of Amblyoponinae genera was well supported in an earlier molecular study (*2*).

The monophyly of the Leptanillinae was strongly supported (100% bpp and ml bs), and its basal position was recovered in all analyses. Ward (*6*) noted that a basal position of *Leptanilla* within the poneroid group implies that tergosternal fusion of abdominal segments III and IV in the worker caste occurred early in ant evolution and was lost secondarily in many lines. Our results indicate that these characters are indeed labile and homoplasious. Although the basal position of Leptanillinae was suggested in other molecular studies (*2*, *10*), previous phylogenetic hypotheses based on morphology had failed to place it in a basal position among extant ants.

Bolton (*1*) proposed a "poneromorph" clade, including Amblyoponinae, Ectatomminae, Heteroponerinae, Paraponerinae, Ponerinae, and Proceratiinae; our results exclude Ectatomminae and Heteroponerinae but add Agroecomyrmecinae. The latter is represented by a single extant species, *Tatuidris tatusia*, and two fossil genera, and its placement within the poneroid clade is entirely novel. Both Ectatomminae and Heteroponerinae nested within the formicoid clade. Although the poneroid clade received less support in the maximum likelihood and maximum parsimony analyses (Table 1), it was strongly supported in the Bayesian analysis (100% bpp). It seems likely that the five included subfamilies form a monophyletic group or, alternatively, a basal polytomy, but in either case they remain outside both the leptanilloid and the formicoid clades.

The inclusion of Heteroponerinae within the formicoid clade is also unexpected. As suggested by their name, heteroponerines have historically been placed in the poneromorph clade. Moreover, Ectatomminae, until recently also considered poneromorphs, appear to be closely related to Heteroponerinae. These findings, combined with the lack of stability for the "poneromorphs" observed in morphological analyses (*4*–*7*), underscore the extent to which our understanding of ancestral ant morphology and behavior must be revised.

The phylogenetic position of *Aneuretus*, today restricted to Sri Lanka, has been hypothesized to be basal either to the Dolichoderinae or to the Dolichoderinae + Formicinae (*12*, *13*). We recover *Aneuretus* as basal to the Dolichoderinae, with both groups separated from Formicinae, implying that the sting has been reduced independently at least twice in the ants (Dolichoderinae and Formicinae).

The ant fossil record is extensive, with more than 60 extant and 100 extinct genera. The oldest reliably dated fossils are ~100 million years (My) old, from Early Cretaceous French and Burmese ambers (*14*, *15*). These include both *Gerontoformica* and *Burmomyrma* (Aneuretinae), with features typical of modern "crown group" ants, as well as Sphecomyrminae, with features typical of basal "stem group" ants. Although no older sphecomyrmines are known, the presence of stem and crown group ants in these roughly coeval ambers implies an earlier history of Formicidae. The status of the Armaniinae/-idae as stem group ants is controversial (*1*, *3*, *15*), but if they are viewed as sister to Formicidae, this also implies an extension of the minimum age of ants to the maximum age of Armaniinae/-idae, which has been estimated to be ~125 My (*16*).

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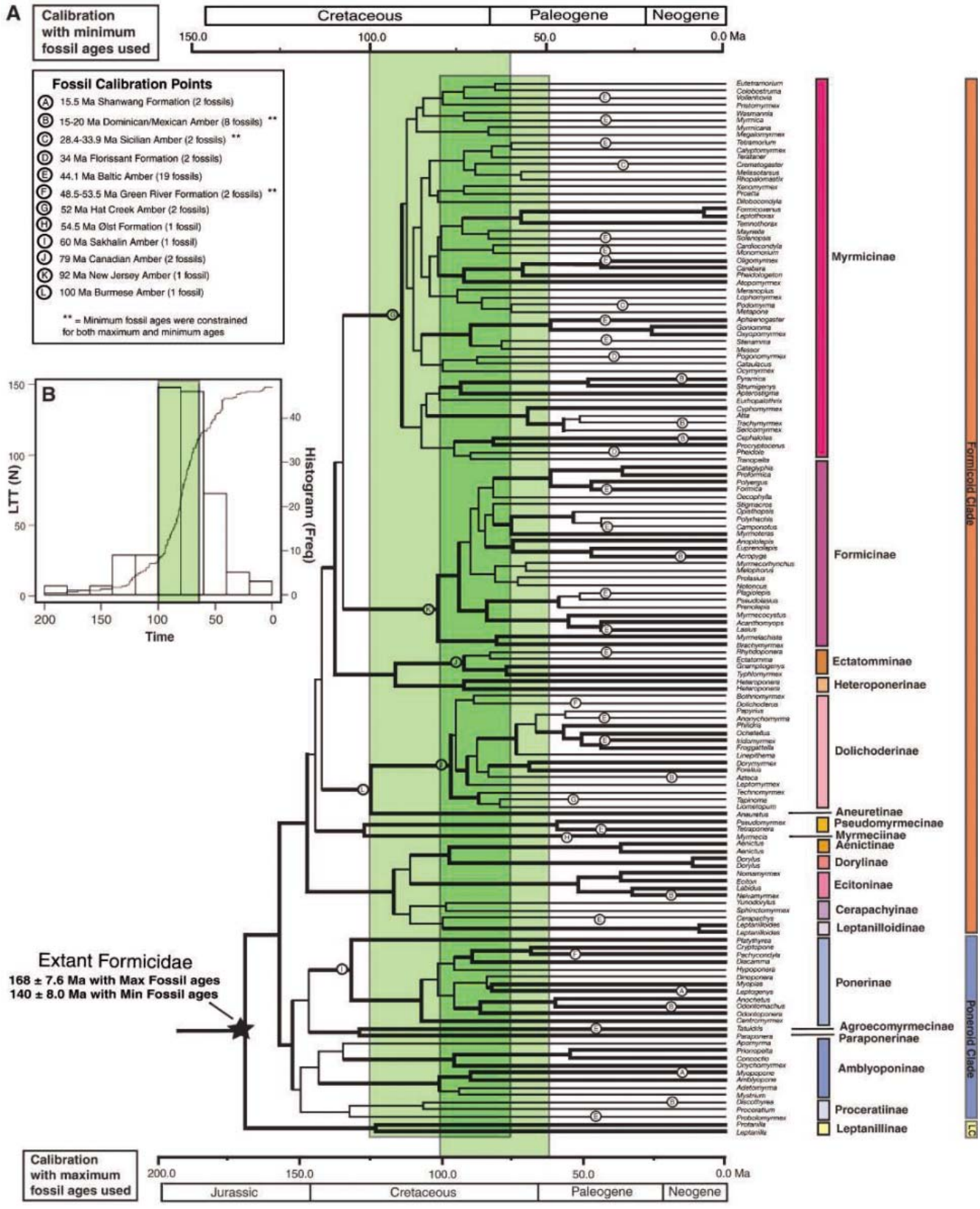

Fig. 1. Phylogenetic chronogram of ants (**A**). Proportional lineages-through-time (LTT) plot for ants and distribution of clade ages for crown group ants (**B**). Bayesian posterior probability clade support of 100% is indicated by thick lines on topology. Fossil calibration points are indicated on topology. Shaded areas (green) delineate the rise of angiosperm dominance depending on whether minimum or maximum ages are used for fossil calibrations (respective time scales are indicated above and below topology), and the overlap between these two regions corresponds to the greatest period of ant diversification. The LTT plot for ants shows the number of lineages present (as a proportion of terminals) at sequential time points. The shaded area (green) on the LTT plot and histogram likewise correspond to the overlapping region of the origin of angiosperm-dominated forests [(A) and (B)].

Table 1. Clade support for phylogenetic analyses and divergence time estimates. Support for clades recovered under Bayesian posterior probabilities, maximum likelihood bootstrap, and maximum parsimony bootstrap (bpp/ml bs/mp bs). Divergence time estimations ±1.96 SD of the bootstrapped samples. *, not applicable because only one taxon represents clade or outgroup; –, support less than 50%; †, minimum age of fossils used as calibration points (otherwise, maximum age of fossils used as calibration points); nm, not monophyletic; PL, penalized likelihood.

Clade	Support for combined analyses	Support for no missing data analyses	Support for Bayesian mixed model analysis	PL divergence time estimates	PL† divergence time estimates
Myrmicinae	100/100/100	100/100/98	100	114.0 ± 4.5	99.8 ± 4.2
Formicinae	100/100/100	100/100/100	100	101.4 ± 3.8	92.0 ± 0.2
Ectatomminae	100/100/98	100/100/95	100	92.3 ± 0.6	79.5 ± 0.9
Heteroponerinae	100/100/100	*/*/*	100	91.8 ± 2.7	79.0 ± 3.0
Dolichoderinae	100/100/98	100/100/98	100	96.6 ± 1.9	85.6 ± 2.2
Aneuretinae	*/*/*	*/*/*	*	124.6 ± 4.8	107.7 ± 5.4
Pseudomyrmecinae	100/100/100	100/100/100	100	59.2 ± 0.9	50.5 ± 1.2
Myrmeciinae	*/*/*	*/*/*	*	127.2 ± 2.2	108.3 ± 3.0
Aenictinae	100/100/100	*/*/*	100	37.3 ± 0.8	31.6 ± 1.1
Dorylinae	100/100/100	100/100/100	100	12.0 ± 0.4	10.2 ± 0.7
Ecitoninae	100/100/100	100/100/100	100	52.1 ± 1.0	44.2 ± 2.0
Cerapachyinae	76/–/–	*/*/*	–	nm	nm
Leptanilloidinae	100/100/100	*/*/*	100	9.7 ± 0.4	8.0 ± 0.3
Ponerinae	100/77/–	100/97/–	100	131.5 ± 5.9	110.7 ± 6.3
Agroecomyrmecinae	*/*/*	*/*/*	*	128.7 ± 4.5	108.2 ± 5.0
Paraponerinae	*/*/*	*/*/*	*	128.7 ± 4.5	108.2 ± 5.0
Amblyoponinae	–/–/–	–/–/–	100	143.1 ± 5.2	113.3 ± 4.9
Proceratiinae	89/71/–	*/*/*	99	131.9 ± 3.9	111.0 ± 3.5
Leptanillinae	100/100/100	100/100/100	100	123.0 ± 3.4	102.4 ± 4.1
Formicoid clade	100/100/93	100/55/93	100	147.0 ± 8.2	124.7 ± 6.5
Poneroid clade	100/64/–	100/97/–	95	152.4 ± 6.2	128.2 ± 5.9
Leptanilloid clade	100/94/60	100/87/65	100	123.0 ± 3.4	102.4 ± 4.1
Formicidae	100/100/100	*/*/*	100	168.8 ± 7.6	140.6 ± 8.0

Because the stratigraphic positions of some fossils in our analyses are not resolved within their dated formations, we conducted all analyses with both maximum and minimum ages for those fossils (Table 1). Our divergence time estimates (*11*) suggest that crown group ants last shared a common ancestor during the Early Cretaceous to Middle Jurassic: 140 ± 8.0 million years ago (Ma) (using minimum ages) to 168 ± 7.6 Ma (using maximum ages) (Fig. 1A). This is considerably older than the ~125-My age estimate based on fossil data. Our findings partially overlap with those of Crozier *et al*. (*17*), who used about six taxa and mitochondrial sequence data to estimate the age of Formicidae at 185 ± 36 My.

Brady (*18*) and Ward and Brady (*19*) used molecular clock evidence to arrive at an age estimate of 130 to 140 My for crown group ants. Their studies were primarily aimed at dating specific lineages and sampled a limited number of fossils to provide minimum age calibration points. Our dates for the origin of the army ant clade (~110 Ma) are similar to those in Brady's study, but the inclusion of wider sampling and additional fossils leads us to an older estimate for the origin of extant ants.

From our analyses (*11*), we find that much of the diversification of the major ant lineages (Fig. 1A) occurred from the beginning of the Early Paleocene to the Late Cretaceous, 60 to 100 Ma, with ancestors of the major subfamilies present as early as 75 to 125 Ma. The fossil record, however, indicates that ants were relatively rare in the Cretaceous, with their march toward ecological dominance only beginning in the Eocene; they are represented by more than 90 species in ~45 genera in Baltic amber, including many extant genera (*15*, *20*). Our data suggest that most of the subfamilies representing extant ants arose much earlier than previously proposed but only began to diversify during the Late Cretaceous to Early Eocene. If ancestors of the major subfamilies were present as early as 75 to 125 Ma, why were they so slow to diversify?

We infer that the rise in angiosperm-dominated forests was harbinger to the diversification of the ants. The window encompassing angiosperm dominance shifts on our chronogram depending on whether we accept the minimum or maximum ages for the ant fossil calibration points (Fig. 1A, shaded green areas). A lineage-through-time (LTT) plot shows a dramatic accumulation of ant lineages at ~100 Ma, either toward the end or immediately following the radiation of the angiosperms (Fig. 1B). These analyses indicate that ant diversification closely tracks the rise of angiosperm-dominated forests, between the Early Paleocene and the Late Cretaceous, 60 to 100 Ma (*21*–*24*). The proliferation of angiosperms is thought to have driven the diversification of major herbivorous groups such as beetles (*25*, *26*) and hemipterans (*16*), and it would appear that ant diversification, too, closely tracks the rise of angiosperm-dominated forests.

At least two explanations could account for these correlated patterns of diversification, although other, as yet unidentified causative factors may have been involved. First, the litter of angiosperm forests is more diverse, providing a wider array of habitats. Modern ant diversity is highest in the soil and ground litter of the world's angiosperm forests, particularly in the tropics (*27*). Second, the expansion of herbivorous insects provided both a direct food resource for hunting ants and an indirect one in the form of honeydew and larval secretions that "agricultural" ants could harvest. A substantial proportion of arboreal ants in modern Amazonian forests have been found to feed on secretions from Hemiptera and extrafloral nectarines (*28*, *29*). In their dynastic-succession hypothesis of ant evolution, Wilson and Hölldobler (*27*) similarly stressed the importance of complex habitats provided by angiosperms and the transition from predation to harvesting secretions (*16*). Presumably this shift in diet also contributed to the evolution of associated social behaviors necessary to exploit and defend these food resources.

A robust hypothesis for the phylogeny of ants permits evolutionary investigation of life history, ecology, and biogeography in generating observed patterns of distribution and diversification of one of the most dominant animal groups. Our phylogenetic and molecular clock analyses of DNA from ants indicate that ants began to diversify much earlier than previously hypothesized and that the rise of the angiosperms may have directly influenced the diversification of this group. Since the mid-Mesozoic, ants have become the insect world's major predators, scavengers, and mutualists. Despite their dominance, we are only beginning to appreciate factors shaping the evolution of this group, highlighting the need for conservation of habitats harboring ant biodiversity, as well as further research on those lineages with poorly understood life histories.

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Supporting Online Material

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Platelet-Derived Serotonin Mediates Liver Regeneration

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The liver can regenerate its volume after major tissue loss. In a mouse model of liver regeneration, thrombocytopenia, or impaired platelet activity resulted in the failure to initiate cellular proliferation in the liver. Platelets are major carriers of serotonin in the blood. In thrombocytopenic mice, a serotonin agonist reconstituted liver proliferation. The expression of 5-HT2A and 2B subtype serotonin receptors in the liver increased after hepatectomy. Antagonists of 5-HT2A and 2B receptors inhibited liver regeneration. Liver regeneration was also blunted in mice lacking tryptophan hydroxylase 1, which is the rate-limiting enzyme for the synthesis of peripheral serotonin. This failure of regeneration was rescued by reloading serotonin-free platelets with a serotonin precursor molecule. These results suggest that platelet-derived serotonin is involved in the initiation of liver regeneration.

Serotonin (5-hydroxytryptamine, 5-HT) is not only a neurotransmitter but also a hormone with various extraneuronal functions (*1*). It is a potent mitogen and modulates the remodeling of tissue (*2*–*5*). Platelets (thrombocytes) carry serotonin in the blood and release it at sites of tissue injury as part of their action on hemostasis (*6*–*8*). However, platelets are also involved in the inflammatory reaction after tissue injury, which is independent of coagulation (*9*). In the liver, platelets interact with leukocytes in response to cold ischemia and induce them to adhere to the endothelium of blood vessels, thereby enhancing tissue injury (*10*, *11*). Concurrent activation of liver macrophages called Kupffer cells leads to further endothelial cell damage and hepatocyte apoptosis (*12*). Depending on the extent of initial tissue injury, the liver can regenerate in a highly synchronized and organized fashion. Because platelets interact with endothelial cells in the early phase after injury, they might also have an effect on the initiation of liver regeneration.

To establish the role of platelets and their secretory products in liver regeneration, partial hepatectomy was performed in mice in which platelet function was inhibited pharmacologically or platelets were depleted. Initially, thrombocytopenia was induced by injecting busulfan, an alkylating agent that causes massive loss of platelets (*13*). Furthermore, platelets were functionally targeted by the application of clopidogrel, which selectively and irreversibly antagonizes the P2Y12 adenosine diphosphate (ADP) receptors on platelets, leading to the inhibition of platelet aggregation (*14*). After injection of these drugs in mice, a 70% hepatectomy was performed to study regeneration of the liver. Although control animals reacted with an increase in hepatic proliferation [5-bromo-2′-deoxyuridine (BrdU)–, Ki67-, and proliferating cell nuclear antigen (PCNA)–positive] 2 days after hepatectomy, busulfan-injected mice exhibited a reduced response (Fig. 1, A to C, and E). In busulfan-treated mice, the number of platelets was reduced in a dose-dependent fashion and the leukocyte count was decreased, but erythrocytes were unaffected (Fig. 1D). Thus, these mice exhibited a combined thrombocytopenia and leukopenia. The impairment of hepatocyte proliferation after hepatectomy may be attributed to a lack of each cell type alone or a combination of both.

To investigate the role of platelets more selectively, an antibody to GPIbα recognizing an epitope on platelets was injected into mice before hepatectomy (*15*). The number of platelets fell below 10% (Fig. 2A), whereas leukocyte and erythrocyte counts were not affected (Fig. 2, B and C), indicating a specific thrombocytopenia. After 70% hepatectomy, all markers of hepatocellular proliferation were reduced (Fig. 2, D to F) in thrombocytopenic mice.

We also tested whether the inhibition of platelet activity, without affecting the number of platelets, was sufficient to block liver regeneration. Clopidogrel, which inhibits the aggregation response to ADP without affecting platelet stability, reduced hepatocyte proliferation in partially hepatectomized livers, but this effect was less pronounced than in busulfan-treated mice. In mice treated with an enantiomer of clopidogrel, which lacks antiaggregation properties, proliferation was not different from controls (Fig. 1, A to C).

Platelets store and release serotonin. About 95% of all serotonin found in blood is stored in platelets. In vitro, serotonin is a potent mitogen and stimulates hepatocyte mitosis (*3*, *16*). The 5-HT2A and 1C receptors appear to mediate mitogenic effects in fibroblasts (*17*, *18*), and the 5-HT2B receptor is involved in the development of the heart (*19*) and the enteric nervous system (*20*). To test whether serotonin induces hepatocyte proliferation in vivo, thrombocytopenic mice were treated with the serotonin receptor 5-HT(2A/2C) agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI-hydrochloride). The application of this drug had no effect on the extent of thrombocytopenia (Fig. 2G) induced by concurrent treatment with the antibody to GPIbα. In the presence of the serotonin agonist, proliferation was completely restored (Fig. 2, D to F).

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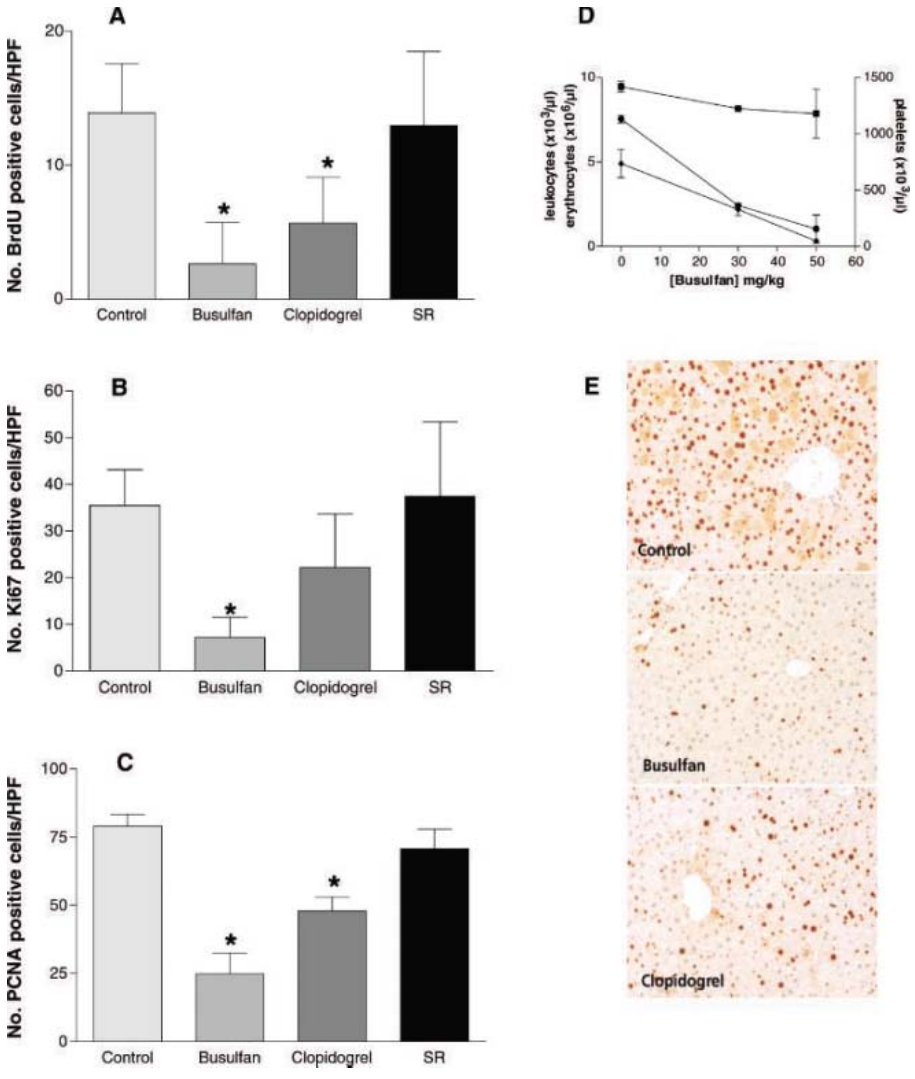

Fig. 1. Effect of drugs targeting platelets on liver regeneration. (**A**) Number of BrdU-positive hepatocytes 2 days after hepatectomy in the remnant liver of control animals and animals treated with either busulfan (thrombocytolytic), clopidogrel (ADP receptor antagonist), or SR25989 (SR) (clopidogrel enantiomer). One-way analysis of variance (ANOVA) was significantly different from 1 ($P = 0.0002$) in the number of BrdU-positive cells in busulfan- and clopidogrel-treated animals. A post-test using Bonferroni comparison exhibited statistical differences between control and busulfan ($P < 0.001$), and between control and clopidogrel ($P < 0.05$) (indicated by asterisk). (**B**) Number of Ki67-positive hepatocytes in control, busulfan-, clopidogrel-, or SR-treated animals. The same remnant livers were used for this analysis. One-way ANOVA indicated a statistical difference ($P = 0.003$) and the Bonferroni post-test indicated a significant difference between the controls and busulfan-treated animals (asterisk). (**C**) Number of PCNA-positive cells in the same remnant liver lobes. One-way ANOVA indicated a difference with $P = 0.0001$, whereas both busulfan ($P < 0.001$) and clopidogrel ($P < 0.01$) were different from controls (asterisk). (**D**) Determination of blood cell counts in animals treated with busulfan. Fifteen days after a single intraperitoneal (ip) injection of busulfan, blood was drawn to determine the number of platelets (circles), leukocytes (diamonds), and red blood cells (squares). Initially, time curves and dose-response curves were evaluated to determine the optimal time and concentration of busulfan. In a second set of experiments, the number of blood cells was evaluated by using two concentrations of busulfan [30 mg busulfan per kg mouse mass (mg/kg) and 50 mg/kg] compared with the vehicle control. (**E**) Remnant liver sections immunohistochemically stained for PCNA 2 days after hepatectomy. Representative sections of controls (top panel), busulfan-injected (center panel), and clopidogrel-treated mice (bottom panel) are shown. Error bars indicate standard deviation.

To identify the putative 5-HT receptors involved in liver regeneration, we analyzed the expression of the various 5-HT receptor types in the resting liver using real-time polymerase chain reaction with probes recognizing transcripts coding for 1A, 1B, 1D, 1F, 2A, 2B, 2C, 3A, and 3B serotonin receptor types. In the naïve liver, RNA encoding of all receptor types was detectable, except for 1F and 2C. Two days after hepatectomy, a three- to fourfold up-regulation of 2A and 2B receptor expression was observed, suggesting that type 2A and 2B receptors contribute to liver regeneration (Fig. 2H). Mice were treated with specific 5-HT receptor antagonists and submitted to partial hepatectomy. When a 5-HT2A receptor antagonist was used, hepatocyte proliferation measured by Ki67 and PCNA (Fig.2I) staining was reduced, compared with vehicle-treated controls. The 5-HT2B receptor antagonist caused a reduced Ki67 staining, whereas PCNA was not different. Neither 5-HT2C nor 5-HT3 receptor antagonists reduced the labeling indexes (Fig. 2, I and J). These experiments suggest that 5-HT2A and 2B receptor subtypes mediate serotonin-dependent regeneration.

The reconstitution of hepatocyte proliferation by a serotonin agonist suggests that serotonin might be a comitogen in liver regeneration. To further test this hypothesis, we used knock-out mice that lack peripheral serotonin but retain neural serotonin action (*21*). In wild-type animals, tryptophan is converted to 5-hydroxytryptophan (5-HTP) by tryptophan-hydroxylase 1 (TPH1) in the small intestine (fig. S1). In a further step, 5-HTP is converted to 5-HT by a ubiquitous aromatic L–amino acid decarboxylase (*22*). Serotonin is then transferred to platelets by a transporter system. In TPH1$^{-/-}$ mice, platelets lack serotonin (*22*), which is confirmed in platelet-rich plasma (Fig. 3A). To test directly the function of serotonin in liver regeneration, we performed partial hepatectomy on TPH1$^{-/-}$ and wild-type control mice. In hepatectomized TPH1$^{-/-}$ mice, all markers of hepatocyte proliferation were reduced (Fig. 3, B to D) 2 days after hepatectomy. To exclude the possibility that liver regeneration is delayed and not impaired in TPH1$^{-/-}$ mice, hepatocyte proliferation was also analyzed 1 and 4 days after hepatectomy. Neither wild-type nor TPH1$^{-/-}$ mice exhibited proliferative activity at those time points, supporting the idea that the peak of regeneration is at 2 days and that the reduction of hepatocyte proliferation was not caused by a temporal shift (Fig. 3F). This result suggests that a molecular action of serotonin was involved in the induction of hepatoctye proliferation after a major loss of hepatic tissue.

To further substantiate the mitogenic activity of serotonin, TPH1$^{-/-}$ mice were injected with the serotonin precursor 5-HTP to reload their platelets. In these mice, platelets carried completely reconstituted levels of serotonin (Fig. 3A), and all markers of proliferation were restored after partial hepatectomy (Fig. 3, B to E). In addition, livers of TPH$^{-/-}$ mice showed a reduction of mitotic figures, whereas after reloading with 5-HTP, the number reached those of wild-type animals. Similarly, the 5-HT2A antagonist treatment reduced mitotic figures (fig. S2). Thus, serotonin is pivotal for hepatic proliferation after a major tissue loss.

We demonstrated a block of hepatocyte proliferation in thrombocytopenic mice and after clopidogrel treatment, which inhibits platelet func-

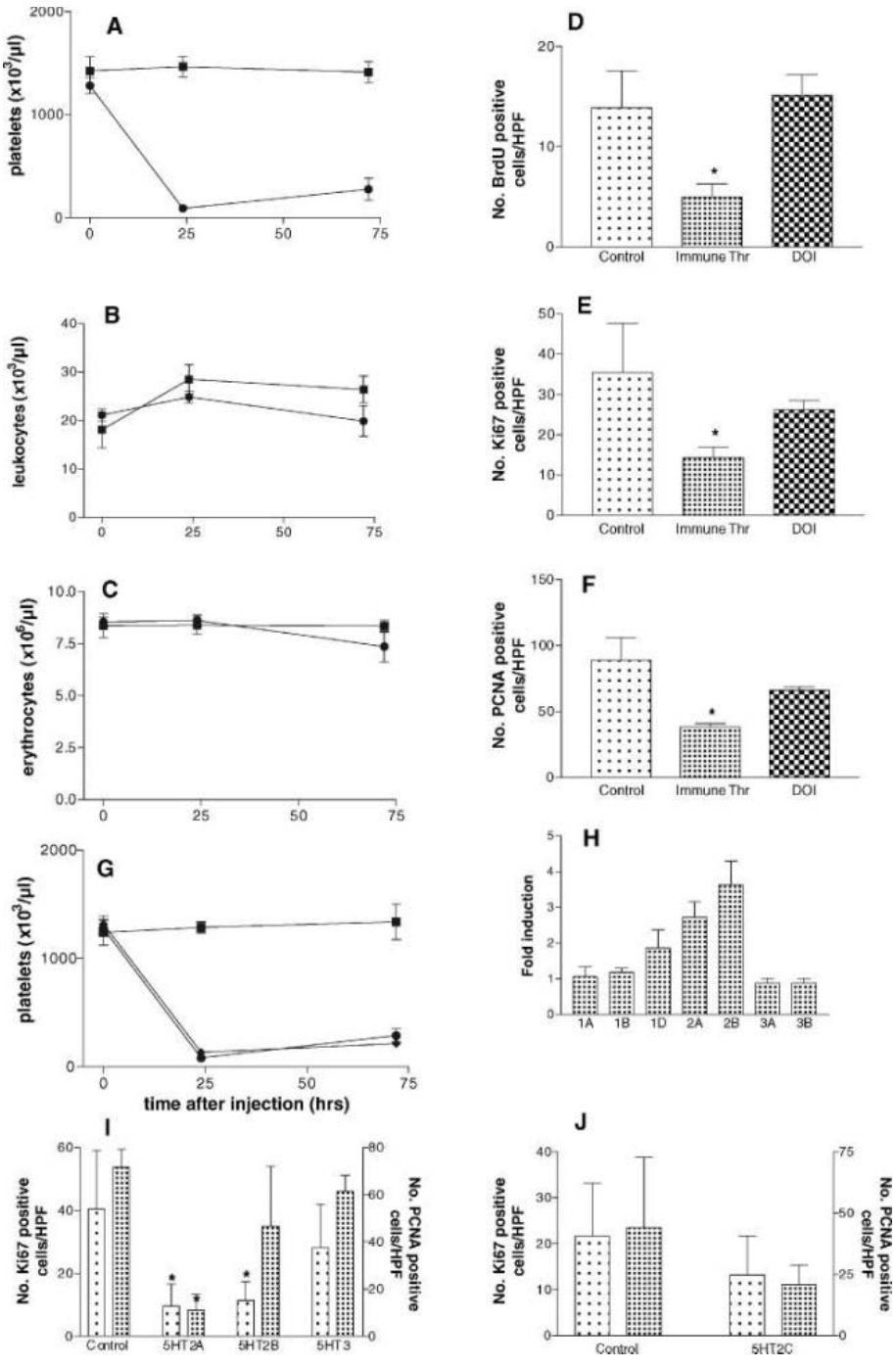

Fig. 2. Effect of thrombocytopenia on liver regeneration. Mice were injected ip with an antibody directed against a platelet epitope, GPIbα, or an immunoglobulin G2 (IgG2) control at 0 and 24 hours. To monitor cell counts, blood was drawn at 0, 24, and 72 hours after the initial injection. (**A**) Number of platelets in anti-GPIbα–treated (circles) and control animals (squares). (**B** and **C**) Number of leukocytes and erythrocytes in the same animals. (**D**) Effect of 70% hepatectomy on proliferation of hepatocytes in the remnant liver 2 days later. BrdU-positive cells were counted in controls, thrombocytopenic (anti-GPIbα), and thrombocytopenic mice treated with the serotonin agonist DOI. (**E**) Number of Ki67 positive cells in the same specimen. (**F**) Detection of PCNA in controls, thrombocytopenic, and DOI-treated animals. In (D) to (F), one-way ANOVA indicated a statistical difference ($P < 0.003$) and the Bonferroni post-test indicated that the controls and the DOI-treated animals were significantly different from the anti-GPIbα–treated animals (asterisk). (**G**) To determine whether the serotonin agonist had any effect on platelet number, thrombocytopenic animals were injected with DOI and bled according to the scheme depicted above. Thrombocytopenic (circles), control animals (squares), DOI (diamonds). (**H**) Induction of transcripts coding for 5-HT receptor subtypes 1A, 1B, 1D, 1F, 2A, 2B, 2C, 3A, and 3B. Subtypes 1F and 2C were below the limit of detection and are not included. The levels of mRNA expression in resected tissue was used as the baseline to calculate the fold induction of transcripts after regeneration. (**I** and **J**) Effect of serotonin antagonists on liver regeneration. Antagonists were injected before and during the period of regeneration. Subtype-specific antagonists are: ketanserin (5-HT2A), SB 206553 (5-HT2B/2C), SB 242084 (5-HT2C), and odansetron (5-HT3). Controls included saline injections, and for 5-HT2C, a separate series with the solvent for SB 242084. Error bars indicate standard deviation.

tion (*23*). These results suggest that platelets play an essential role in liver regeneration. In addition to the function of platelets in coagulation, reperfusion injury in a number of organs (*24*–*28*) [including the liver (*10*, *11*)], and in inflammatory processes (*9*) [such as asthma (*29*), atherosclerosis (*30*), and sepsis (*31*)], the function of platelets in liver regeneration appears to be mediated by serotonin, because mice lacking platelet serotonin displayed lack of liver regeneration. Moreover, serotonin agonists indicate that serotonin may act downstream of a potential interaction of platelets and leukocytes with endothelial cells or hepatocytes. Serotonin has been shown to exert mitogenic actions on smooth muscle cells and fibroblasts in pulmonary hypertension (*32*–*34*) and to modulate the plasticity of the nervous system (*1*, *4*) and the mammary gland (*5*).

The presence of serotonin receptor subtypes 5-HT2A and 2B in the liver, combined with the observation that 5-HT antagonists inhibit liver regeneration, suggests that serotonin acts directly in the liver and not through a remote systemic pathway. The presence of 5-HT2A and 2B in hepatocytes (*35*), which form the parenchymal mass in the liver, is in line with our observation of impaired hepatocyte proliferation after depletion of serotonin or after antagonist treatment.

Both platelets and leukocytes derived from circulating blood, as well as the presence of Kupffer cells, are required for a full hepatic response (*36*). Because hepatic regeneration may occur under different situations, different mechanisms may come into action to rescue liver failure. It has been demonstrated that Kupffer cell–derived cytokines may play a key role in initiating proliferation (*37*). Kupffer cells are predominantly activated during an ischemic insult. The release of the proinflammatory tumor necrosis factor α (TNFα) (*38*) and interleukin-6 (IL-6) (*39*) have been studied in circumstances when tissue damage by apoptosis and necrosis are observed.

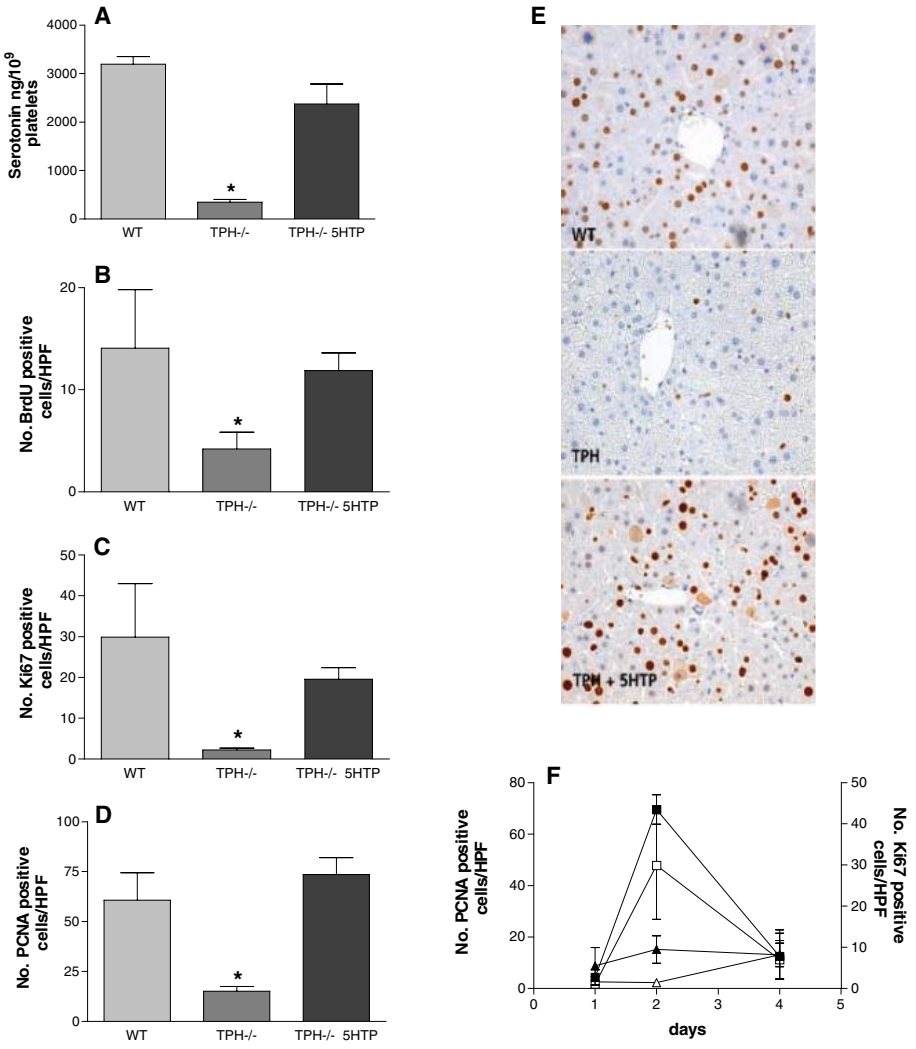

Fig. 3. Effect of serotonin on liver regeneration. (**A**) Serotonin levels in thrombocytes of wild type (WT) mice, TPH1$^{-/-}$ mice, and TPH1$^{-/-}$ mice after supplementing with 5-HTP (TPH1$^{-/-}$ 5-HTP). Platelet-rich plasma was prepared and, after counting platelet concentration, serotonin was assessed by an enzyme-linked immunosorbent assay. One-way ANOVA indicated a statistical difference ($P < 0.001$) and the Bonferroni post test indicated that WT animals ($P < 0.001$) and the TPH1$^{-/-}$ 5-HTP animals ($P = 0.001$) were significantly different from the TPH1$^{-/-}$ animals (asterisk). (**B**) Effect of serotonin depletion in platelets on hepatocyte proliferation 2 days after hepatectomy. The number of BrdU-positive cells was counted in WT, TPH1$^{-/-}$, and TPH1$^{-/-}$ mice supplemented with 5-HTP (TPH1$^{-/-}$ 5-HTP). (**C** and **D**) Number of Ki67- and PCNA-positive cells in the same liver remnants. In [(B) to (D)], one-way ANOVA indicated a statistical difference ($P < 0.01$) and the Bonferroni post-test indicated that the controls and the TPH1$^{-/-}$ 5-HTP animals were significantly different from the TPH1$^{-/-}$ animals (asterisk). (**E**) Histological examples of PCNA-stained sections from remnant livers. (**F**) Time course of labeling indexes for PCNA (solid square and solid triangles) and Ki67 (open squares and open triangles) in hepatectomized wild-type (solid and open squares) and TPH$^{-/-}$ livers (solid and open triangles). Error bars indicate standard deviation.

These cytokines determine the initial induction of proliferation. However, another factor—hepatocyte proliferation factor—appears crucial to the completion of regeneration, whereas maintenance of proliferation is not dependent on these factors. Thus, several factors appear to be involved in proliferation that are not necessarily all required to be present at the same time. Platelet-derived serotonin may influence the proliferation of hepatocytes (*16*) or may be involved in the release of growth factors, such as IL-6, at the site of liver injury (*40*, *41*). These findings have direct clinical implications. In liver transplantation, most patients have reduced platelet counts related to portal hypertension and hypersplenism and, thus, serotonin agonists may be a therapeutic option to improve the outcome.

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The Competitive Advantage of Sanctioning Institutions

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Understanding the fundamental patterns and determinants of human cooperation and the maintenance of social order in human societies is a challenge across disciplines. The existing empirical evidence for the higher levels of cooperation when altruistic punishment is present versus when it is absent systematically ignores the institutional competition inherent in human societies. Whether punishment would be deliberately adopted and would similarly enhance cooperation when directly competing with nonpunishment institutions is highly controversial in light of recent findings on the detrimental effects of punishment. We show experimentally that a sanctioning institution is the undisputed winner in a competition with a sanction-free institution. Despite initial aversion, the entire population migrates successively to the sanctioning institution and strongly cooperates, whereas the sanction-free society becomes fully depopulated. The findings demonstrate the competitive advantage of sanctioning institutions and exemplify the emergence and manifestation of social order driven by institutional selection.

The uniqueness of human cooperation necessitates investigations that reach beyond the explanations of cooperative behavior of nonhuman animals (*1*–*5*). Profound empirical evidence shows that the possibility of sanctioning norm violators stabilizes human cooperation at a high level, whereas cooperation typically collapses in the absence of sanctioning possibilities (*6*–*11*). Would a sanctioning institution deliberately be adopted when individuals can choose between a sanctioning and a sanction-free institution? The considerable payoff losses in the process toward stable cooperation—for both the punishers and the punished individuals—as well as natural resentments against punishment caused, for example, by its detrimental effects (*12*) might guide individuals' choice toward the sanction-free institution.

The argument that higher cooperation levels in sanctioning institutions "automatically" lead to their prevalence—because rational individuals choose the institution with the higher payoff (*13*)—is often brought forward as an affirmative argument for the competitive advantage of sanctioning institutions. The force of this argument can be questioned, however, because it displaces rather than solves the evolutionary puzzle of human cooperation. The reason for this is that stable cooperation requires a positive share of individuals who carry personal costs for cooperation and punishment to the benefit of the entire group (*14*–*16*). These individuals have a clear payoff disadvantage compared to cooperators who free-ride on the punishment acts. Recent research shows that a positive share of strong reciprocators—cooperating individuals who are willing to reward fair behavior and to punish unfair behavior even when they cannot gain materially from doing so—can be evolutionarily stable (*17*, *18*). But what happens if the population is perfectly mobile and is permanently invaded by outsiders from a noncooperative environment who are attracted by high payoffs from cooperation? Is the fraction of strong reciprocators who choose the sanctioning institution sufficiently large to keep up the cooperative culture? These arguments cast serious doubt on the prevalence of sanctioning institutions.

However, several affirmative arguments for the competitive advantage of sanctioning institutions also come to mind, e.g., the large number of institutional frameworks that facilitate the sanctioning of norm violators in human societies (*19*–*21*) and the recent finding that humans derive satisfaction from punishing defectors (*22*). Additionally, theories of cultural and institutional selection (*23*–*26*) that are grounded on the exceptional human ability of social learning support the competitive advantage of sanctioning institutions. They suggest that individuals preferentially migrate to groups with higher payoffs and imitate the decisions prevalent in these groups. Hence, group members punish, because it is common to do so. When cooperation is sufficiently widespread, the payoff-disadvantage from punishing is relatively small, and only a weak tendency for conformist behavior suffices to stabilize the punishment of noncooperators.

We inquire into the competitive advantage of sanctioning institutions in a laboratory experiment in which we implement permanent competition between a sanctioning and a sanction-free institution through endogenous choice. It allows one to study the evolution of the different institutions over time as well as the changes in behavior in the same individual when participating in different social settings.

In our experiment, 84 participants anonymously interact in a social dilemma situation in 30 repetitions. Each repetition consists of three stages: An institution choice stage (S0), a voluntary contribution stage (S1), and a sanctioning stage (S2). In stage S0, the participants simultaneously and independently choose between a sanctioning institution (SI) and a sanction-free institution (SFI) in which neither positive sanctioning (rewards) nor negative sanctioning (punishment) is possible. In stage S1, each participant interacts in a public goods game with all other participants who have chosen the same institution in S0; each player is endowed with 20 money units (MUs) and may contribute between 0 and 20 MUs to a public good. Each group member equally profits from the public good, independent of his or her own contribu-

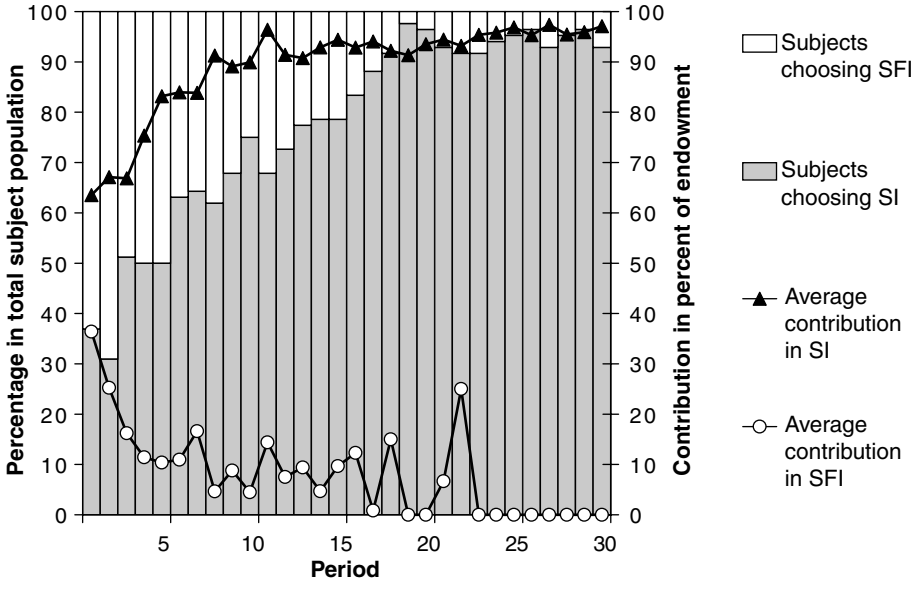

Fig. 1. Subjects' choice of institution and their contributions. The average contributions in both institutions over the 30 periods of the interaction are measured as the percentage of endowment contributed to the public good.

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tion. The MUs not contributed to the public good are transferred to the participant's private account. The diametrically opposed individual and collective interests constitute the social dilemma in public good provision: It is always in the material self-interest of any subject to free-ride on the contributions of others and to keep all MUs for the private account, whereas the collective interest demands full contribution of all group members. After the players have simultaneously made their contribution decisions, they are informed about the contributions of each member in their institution. In stage S2 each player in SI may positively or negatively sanction other members of SI by assigning between 0 and 20 tokens to other members. Each token used as a negative sanction costs the punished member 3 MUs and the punishing member 1 MU. Each token used as a positive sanction yields the receiving member 1 MU and costs the member who uses it 1 MU. At the end of the period each participant receives detailed (but anonymous) information about each of the other participants from both institutions (*27*).

The initial choice of institution provides a clear picture: Only about one-third of the participants (mean = 36.9%; SE = 4.0%) prefer SI to SFI in the first period. The revealed institution preference correlates with different types of behavior (*28*, *29*). Participants who initially join SI contribute on average 12.7 MUs (SE = 0.79) in the first period, while on average only 7.3 MUs (SE = 0.54) are contributed in SFI (Wilcoxon signed rank matched pairs test, $z = -2.366$, $P = 0.016$, two-tailed). Almost half the subjects (mean = 48.4%; SE = 8.5%) who opt for SI in the first period are "high contributors" in that they contribute at least 15 MUs. Almost three-fourths (mean = 73.3%; SE = 17.0%) of these high contributors exert punishment tokens to discipline low contributors and thus try to enforce and establish a norm of high cooperation. These subjects amount to 13.1% (SE = 4.0%) of the total subject population and can clearly be classified as "strong reciprocators," i.e., subjects with a predisposition to make high contributions and to punish norm violators. In contrast, 16.1% (SE = 5.2%) of the subjects in SI contribute 5 MUs or less ("free-riders") in the first period. The situation is completely different in SFI, where in the first period almost half of the subjects are free-riders (mean = 43.4%; SE = 3.4%), whereas high contributors are rare (mean = 11.3%; SE = 4.3%). A subject who chooses SFI in the first period with a contribution of more than 15 MUs and uses negative sanctions immediately after having switched to SI may also be classified as a strong reciprocator. We observed two subjects with this behavior in our subject population (2.4%), so that 15.5% (SE = 5.6%) is a lower bound for the proportion of strong reciprocators in the subject population. Initially, the significantly higher contributions in SI do not result in higher payoffs in SI: Average payoffs in the first period of SI (mean = 38.1 MUs; SE = 2.05) are significantly lower than in SFI (mean = 44.4; SE = 0.32) (Wilcoxon signed rank matched pairs test, $z = -2.047$, $P = 0.047$, two-tailed). Due to frequent punishment activities, free-riders earn significantly less in SI (mean = 30.2; SE = 4.51) than in SFI (mean = 49.7 MUs; SE = 0.86) in the first period (Wilcoxon signed rank matched pairs test, $z = -2.366$, $P = 0.016$, two-tailed).

Although subjects are initially reluctant to join SI, it becomes predominant over time; eventually, nearly all participants (mean = 92.9%; SE = 3.4%) choose SI and cooperate fully (Fig. 1) (*30*). Simultaneously, contributions in SFI decrease to zero. In period 10 the contributions in SI are on average 89.9% (SE = 10.3%) of the endowment and from there on they steadily increase. In the last period the difference between the two institutions is almost as extreme as it can be with average contributions of 19.4 MUs (SE = 0.714) in SI and 0 MUs (SE = 0.0) in SFI. Averaged over all periods, subjects in SI contribute 18.3 MUs (91.4% of the endowment; SE = 5.0%), whereas subjects in SFI contribute only 2.9 MUs (14.4% of the endowment; SE = 3.0%) (Wilcoxon signed rank matched pairs test, $z = -2.366$, $P = 0.016$, two-tailed).

What causes this dramatic change of mind? Pure imitation of the successful behavior would lead to an increase of free-riders in SFI because they earn the highest average payoffs in the first period. This is actually observed in period two. Consequently, the payoffs of free-riders in SFI decrease and over the periods, participants in SFI experience the typically observed collapse of cooperation in repeated social dilemma interactions (Fig. 1). A comparison of the payoffs of the two predominant behavioral patterns—free-riding in SFI and high contributions in SI (Fig. 2)—shows that from period five onward a high contributor in SI achieves a higher payoff than a free-rider in SFI (Wilcoxon signed rank matched pairs test, $z = -2.366$, $P = 0.016$, two-tailed). It therefore pays for a monetary payoff maximizing participant to switch from free-riding in SFI to contributing in SI. This triggers an amplifying effect; namely, the greater the number of cooperators in SI, the higher their payoffs. Indeed, from period 10 onward, 86.1% (SE = 13.1%) of all members of SI contribute fully (20 MUs) and 86.0% (SE = 8.6%) in SFI contribute almost nothing (2 MUs or less). The finding that players apparently choose institutions according to payoffs indicates that stochastic

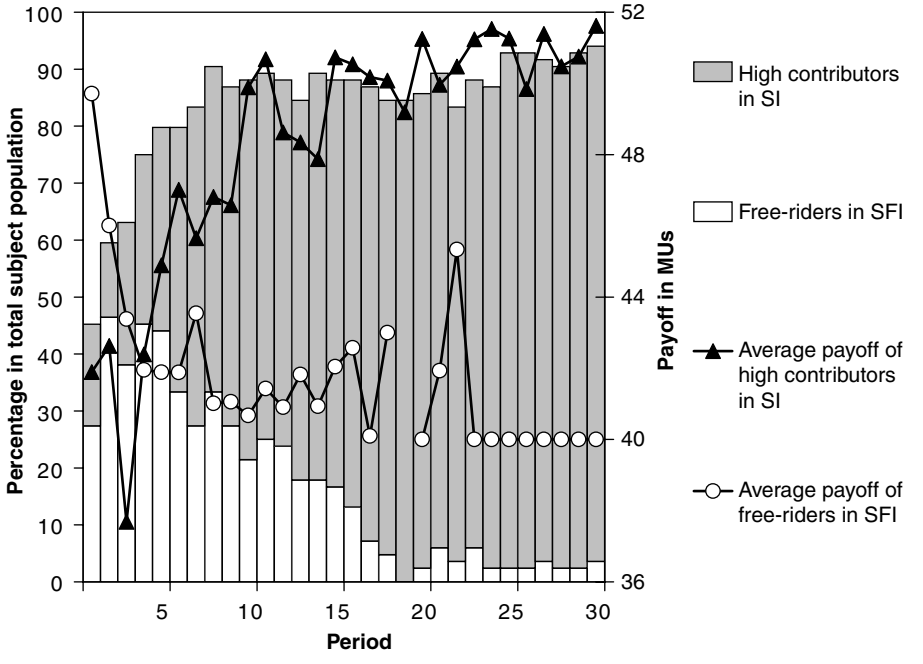

Fig. 2. Payoffs of the two predominant behavioral patterns, "free-riders" (contributions between 0 and 5 MUs) in the sanction-free institution (SFI) and "high contributors" (contributions between 15 and 20 MUs) in the sanctioning institution (SI). The highest attainable payoff (under full contributions of all subjects and no punishment) is 52 MUs and the payoff from complete free-riding and no punishment is 40 MUs.

Table 1. Results of a Tobit regression, independent variable: Contribution ($t + 1$) – Contribution (t). Tobit regression for subjects who opted for SI in period t and ($t + 1$) with a robust estimation for the standard errors using the independent observations as clusters. The values in parentheses denote the robust standard errors.

Independent variable	Coefficient	z value
Negative sanctions in t	0.444 (0.085)	5.24*
Positive sanctions in t	−0.148 (0.102)	−1.45
Constant	0.000 (0.053)	0.00

*Denotes significance at the 1% level.

forces play only a minor role in determining switching behavior (*31*).

A closer look at individual behavior immediately before and after migration from one institution to the other confirms the bipolar pattern of behavior induced by the two institutions. Indeed, 80.3% (SE = 5.0%) of subjects increase their contribution when migrating from SFI to SI in two consecutive periods. Moreover, 27.1% (SE = 5.3%) of subjects even "convert" from being a complete free-rider (contributing 0 MUs) to a full cooperator (contributing 20 MUs) when switching from SFI to SI. The migration behavior in the opposite direction, i.e., from SI to SFI, is similarly extreme. Roughly 70% (mean = 70.9%; SE = 4.9%) of subjects reduce their contribution when switching from SI to SFI and about 20% (mean = 17.0%; SE = 4.7%) switch from full cooperation to free-riding.

Individual payoff maximization cannot explain why new members in SI follow the second norm established by the strong reciprocators who joined SI in early periods, i.e., the norm to punish low contributors. The most successful behavior would be to contribute in SI (and hence avoid being punished), but refrain from the costly punishment of others. Because punishment of defectors constitutes a second-order public good (in which defection cannot be sanctioned in our setting), individual payoff maximization would rule out punishment. However, only a minority of subjects follow this payoff-maximizing behavior. The overwhelming majority of 62.9% (SE = 8.5%) of the subjects immediately conforms to and adopts the prevailing norm of punishment in SI, i.e., they always use punishment immediately after they switch to SI. This results in a quite stable proportion of ~40% (mean = 42.1%; SE = 5.9%) of subjects who both contribute highly and punish during the last 20 periods (Fig. 3). Figure 3 also shows that the payoff difference between high contributors who punish and those who do not constantly diminishes over time because punishment becomes ever more unnecessary. Additionally, because the absolute number of punishers increases, the individual burden from effectively punishing free-riders becomes smaller over time (*32*). Toward the end, subjects who both contribute highly and punish exhibit a payoff disadvantage of less than 2%; hence, the "selection pressure" against strong reciprocators becomes quite weak (*33*). This leads to a continuous increase in efficiency gains in SI up to 95.8% (SE = 4.6%) in the final period, whereas efficiency gains in SFI converge to zero (mean = 0; SE = 0.0).

Although the use of both positive and negative sanctions per individual decreases over time, the ratio in which they are used is rather stable; on average, 1.66 negative sanction points (SE = 0.60) are allocated per positive sanction point. A Tobit regression of the combined effect of positive and negative sanctions exhibits a clear positive impact of punishment on subsequent contributions, whereas positive sanctions have a slightly negative but rather insignificant effect (Table 1). It seems that positive sanctions are not perceived as an unambiguous encouragement to increase the contribution; perhaps they are taken as an indication that the contribution has been higher than expected by others and hence may be lowered. These observations reflect the asymmetry between negative and positive sanctions. Positive sanctions are addressed to those who already abide by the social norm and, to preserve the approval of cooperation, a continuous application of the instrument is required. Negative sanctioning, by contrast, is an instrument for disapproving of norm-violating behavior and need only be exerted if the norm is not followed. If an individual abides by the norm, punishment is not necessary. The threat of punishment alone is able to support cooperation.

Our results show that the sanctioning institution is the undisputed winner in a "voting-with-one's-feet" competition with a sanction-free institution. The results provide profound empirical evidence for the existence and importance of strong reciprocators, as well as a form of conformist behavior, as described in models of cultural selection. The initial establishment of the "norm to cooperate and punish free-riders" is mainly driven by the steadfastness of the strong reciprocators to punish noncooperative subjects, despite severe individual losses (*34*). Although strong reciprocators are a minority, they manage to establish and enforce a cooperative culture that attracts even previously noncooperative individuals and thus resolves the social dilemma. The predominant tendency to punish norm violators after a migration from the noncooperative environment of the sanctioning-free institution to the sanctioning institution provides support for the assumption that humans adapt to the common behavior although it deviates from the payoff-maximizing behavior. This tendency for conformism raises sanctioning activities at a high level such that cooperation can be stabilized.

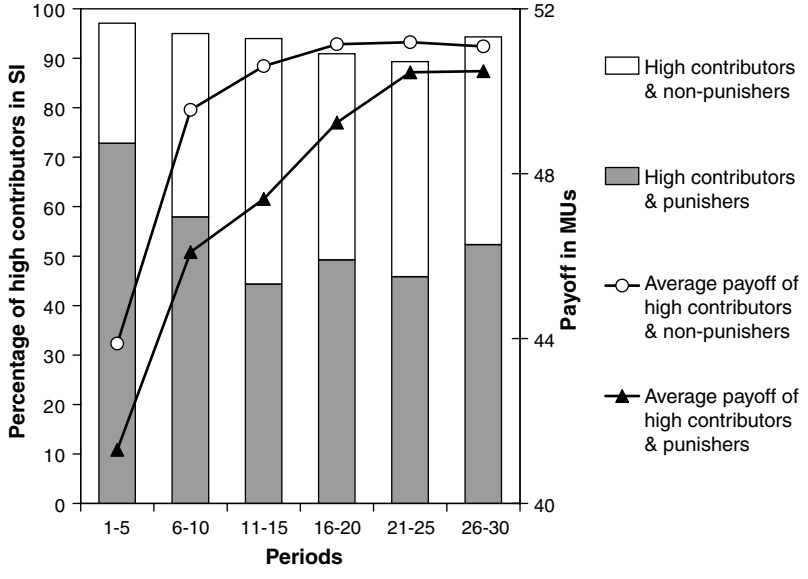

Fig. 3. Payoffs and percentages of punishers and nonpunishers among the "high contributors" (contributions between 15 and 20 MUs) in the sanctioning institution (SI). The highest attainable payoff (under full contributions of all subjects and no punishment) is 52 MUs and the payoff from complete free-riding and no punishment is 40 MUs.

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30. Figure S1 displays the exact flow in both directions between institutions from one period to the next.
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32. A logistic regression shows that the stay duration in SI in terms of the number of periods has a significantly negative influence on the likelihood of punishing others (table S1). Note, however, that individually exerted punishment may be lowered over time to effectively punish a free-rider because the number of potential punishers becomes larger. Indeed, average payoffs of free-riders decrease over periods, as can be seen from fig. S2.
33. In the last 10 periods, subjects who contribute highly and punish reach on average 98.7% of the payoff of subjects who contribute highly but do not punish.
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Darwinian Evolution Can Follow Only Very Few Mutational Paths to Fitter Proteins

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Five point mutations in a particular β-lactamase allele jointly increase bacterial resistance to a clinically important antibiotic by a factor of ~100,000. In principle, evolution to this high-resistance β-lactamase might follow any of the 120 mutational trajectories linking these alleles. However, we demonstrate that 102 trajectories are inaccessible to Darwinian selection and that many of the remaining trajectories have negligible probabilities of realization, because four of these five mutations fail to increase drug resistance in some combinations. Pervasive biophysical pleiotropy within the β-lactamase seems to be responsible, and because such pleiotropy appears to be a general property of missense mutations, we conclude that much protein evolution will be similarly constrained. This implies that the protein tape of life may be largely reproducible and even predictable.

Resistance to β-lactam antibiotics (e.g., penicillin) is commonly mediated by a bacterial β-lactamase, which hydrolytically inactivates these drugs (*1*). Bacterial resistance to novel β-lactams first arises by point mutations in the β-lactamase gene (*2*, *3*). Five point mutations in an allele of this gene that we designate TEM^{wt} (the reference sequence of the TEM family of β-lactamases) (*4*, *5*) jointly increase resistance by a factor of ~100,000 against cefotaxime (*6*, *7*), a third-generation cephalosporin β-lactam. These consist of four missense mutations [A42G, E104K, M182T, and G238S; numbering as in (*8*)] at clinically important residues (*3*, *9*) and one 5′ noncoding mutation [g4205a; numbering as in (*4*)], and we denote this high-resistance quintuple mutant *TEM**. Thus, five mutations must occur for *TEM** to evolve from TEM^{wt}, and because these can in principle occur in any order, there are 5! = 120 mutational trajectories linking these alleles. However, natural selection for heightened cefotaxime resistance may not regard all trajectories equivalently (*10*). Here, we determine the prevalence with which these mutations only conditionally increase drug resistance, a form of interaction previously designated sign epistasis (*10*). Sign epistasis is both necessary and sufficient for one or more trajectories to *TEM** to be selectively inaccessible (*10*).

To characterize the effect on drug resistance of each mutation on all allelic backgrounds, we first constructed the 32 combinations of these five mutations (*11*, *12*). We next determined their resistance to cefotaxime (*12*) in *Escherichia coli* strain DH5α (Table 1); because the sign of the mutational effect on drug resistance determines the selective accessibility of each trajectory (*10*), we also report the rank order of drug resistance values exhibited by all alleles. *TEM** exhibits the highest resistance and, because at least one mutation increases resistance in all other alleles, the fitness landscape is single-peaked (*13*). Thus, in the case of cefotaxime resistance evolution, populations cannot become trapped (*13*) at suboptimal alleles between TEM^{wt} and *TEM**, as was recently also shown for isopropylmalate dehydrogenase (IMDH) evolution from a nicotinamide adenine dinucleotide phosphate (NADP)–dependent form to a nicotinamide adenine dinucleotide (NAD)–dependent form (*14*).

To estimate the relative probabilities with which evolution by natural selection for heightened cefotaxime resistance will realize each of the 120 possible mutational trajectories from TEM^{wt} to *TEM**, we assumed that the time to fixation or loss of individual mutations is far less than the time between mutations [the "strong selection/weak mutation" model of (*15*)]. Thus, the relative probability of realizing any particular mutational trajectory is the product of the relative probabilities of its constituent mutations, because under our assumption the choice of each subsequent fixation is statistically independent of all previous fixations (*12*). Next, for each allele we partitioned all possible mutations into those that are beneficial, deleterious, or neutral with respect to cefotaxime resistance. The probability of

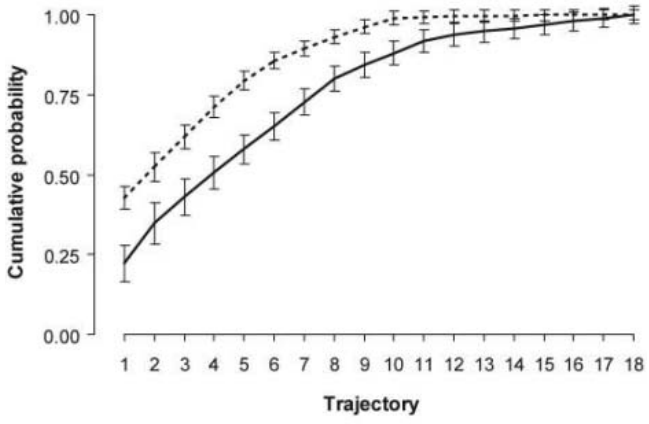

Fig. 1. Estimated cumulative probabilities for all 18 selectively accessible mutational trajectories from TEM^{wt} to *TEM**, under the correlated (broken line) and equal fixation probability (solid line) models, ± SEM. Trajectories are ordered in decreasing probability of realization.

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fixation for a beneficial mutation far exceeds that for deleterious or neutral mutations (*12*, *15*) and, because all alleles have one or more beneficial mutations (Table 1), we approximated the probability of fixation for all other mutations by zero.

Applying this population genetic reasoning (*12*) to the data in Table 1 reveals that 102 of the 120 mutational trajectories from TEM^{wt} to *TEM** are selectively inaccessible. Although these five mutations were chosen for their large joint phenotypic effect, this result is necessarily (*10*) a consequence of the fact that some of the mutations do not increase cefotaxime resistance on all allelic backgrounds. Rather, four mutations have negligible or even negative effects on drug resistance in some combinations. Under our model, the probability of fixation for such mutations on such backgrounds—and hence of those trajectories on which they occur—is zero. The number of alleles on which each mutation has a positive, negative, or negligible effect, together with the mean proportional increase in cefotaxime resistance of each, is reported in Table 2. This illustrates the incidence of sign epistasis in our data set: mutations that only conditionally increase phenotype [(*10*) and supporting online text].

The relative probabilities of realization among the 18 selectively accessible trajectories reflect the probabilities of fixation of their constituent (beneficial) mutations, which in turn depend on the ecological circumstances in which drug resistance evolves (*12*). The simplest model assumes that all available beneficial mutations fix with equal probability [see (*12*) for ecological interpretation]. A biologically more realistic picture assumes that the relative probability of fixation of beneficial mutations is positively correlated with magnitude of effect (*12*), and we employed extreme value theory (*12*, *15*, *16*) to provide intuition into the evolutionary consequences of such a correlation. Extreme value theory provides estimates of these relative probabilities largely independent of the underlying distribution of fitness effects (*16*).

The mean cumulative probabilities of the 18 selectively accessible trajectories under the equal and correlated fixation models are shown in Fig. 1; individual probabilities are presented in table S2. The sharp skew to the left indicates that only a few trajectories capture most of the probability density. For example, half of all evolutionary realizations will follow just four and two trajectories, respectively, under the equal and correlated fixation probability models. Because some correlation between a mutation's effect on resistance and its probability of fixation is likely (*17*), the biologically relevant number is probably closer to the lower value. Note that the results illustrated in Fig. 1 are largely robust to small, undetected differences in drug resistance (see supporting online text). Figure 2 illustrates the source of this bias at the level of the constituent beneficial mutations defining the 10 most probable trajectories, which represent, respectively, ~90% and ~99% of the probability density under the equal and correlated fixation probability models.

The skew in probabilities of realization among trajectories under the equal fixation probability model (Fig. 1) is by definition (*12*) entirely due to the structure of sign epistasis in Table 1. For example, mutations along the most likely trajectory under this model occur in the order G238S, E104K, A42G, M182T, g4205a. Reversing the order of the second and third mutations (G238S, A42G, E104K, M182T, g4205a) defines a second trajectory whose probability of realization under this model is reduced by a factor of three (table S2). This is because after the initial two fixations in the first trajectory, only one beneficial alternative exists, whereas three alternatives exist at that juncture in the second (Fig. 2), reducing the probability of each by one-third (eq. S5a). This effect differs from that due to unequal probabilities of fixation among alternative beneficial mutations (*18*), which gives rise to the modest difference between curves in Fig. 1.

Biochemical and biophysical considerations of β-lactamase offer some insight into the mechanistic origin of the sign epistasis underlying our results. For example, G238S on the TEM^{wt} background is known to enhance cefotaxime hydrolysis (*19*–*21*) but simultaneously to increase aggregation (*19*) and reduce thermodynamic stability of the enzyme (*20*, *22*). Conversely, M182T alone modestly reduces hydrolysis (*20*) while reducing aggregation (*19*) and increasing thermodynamic stability (*20*). Thus, intramolecular pleiotropy of M182T and G238S accounts for the fact that M182T exhibits sign epistasis: On TEM^{wt} it reduces (or at least has negligible effect on) cefotaxime resistance, but together with G238S it increases resistance (Table 1), because the double mutant enjoys increased hydrolysis (*20*) without loss of thermodynamic stability (*23*). The 5′ noncoding mutation g4205a also exhibits sign epistasis. Although it increases gene expression by a factor of ~2.5 (*6*, *7*), the mean effect on resistance of this mutation is much smaller, and it significantly increases resistance in at most 8 of 16 alleles (Table 2). The explanation may involve aggregation of β-lactamase (*23*, *24*): Because

Table 1. Cefotaxime resistance of TEM β-lactamase alleles. Assayed as minimum inhibitory concentration (MIC) (*12*); median value across three replicates shown in μg/ml.

Missense mutations					Without g4205a mutation		With g4205a mutation	
A42G	E104K	M182T	G238S	Clinical designation*	MIC	Rank	MIC	Rank
–	–	–	–	TEM-1	0.088†	13‡	0.088	13
–	–	–	+	TEM-19	1.4	9	1.4	9
–	–	+	–	TEM-135	0.063†§	14	0.088§	13
–	–	+	+	TEM-20	32	6	3.6×10^2	5
–	+	–	–	TEM-17	0.13‖	12	0.18	11
–	+	–	+	TEM-15	3.6×10^2	5	3.6×10^2	5
–	+	+	–	TEM-106	0.18‖	11	0.18	11
–	+	+	+	TEM-52	3.6×10^2	5	2.1×10^3	3
+	–	–	–	None	0.088	13	0.088	13
+	–	–	+	None	23	7	3.6×10^2	5
+	–	+	–	None	1.4	9	0.088	13
+	–	+	+	None	3.6×10^2	5	3.6×10^2	5
+	+	–	–	None	1.4	9	2.0	8
+	+	–	+	None	2.1×10^3¶	3	1.5×10^3¶	4
+	+	+	–	None	0.80	10	1.4	9
+	+	+	+	None	2.9×10^3	2	4.1×10^3	1#

*Of protein sequence; from (*3*). †These two values not significantly different after Bonferroni correction. ‡This allele here designated TEM^{wt}. §These two values not significantly different after Bonferroni correction. ‖These two values not significantly different after Bonferroni correction. ¶These two values not significantly different after Bonferroni correction. #This allele here designated *TEM**.

the fraction of molecules aggregated rises with protein concentration (*25*), missense mutations that reduce aggregation [e.g., (M182T)] (*19*) may be necessary to render g4205a beneficial. (Compare the effects of g4205a on A42G/E104K/G238S with that on A42G/E104K/M182T/G238S in Table 1.) Thus, here again, pleiotropy represents the mechanistic basis of sign epistasis.

Seen as an analysis of clinical cefotaxime resistance evolution, our treatment makes several simplifying assumptions about the mutational and selective processes. For example, we have disregarded horizontal gene transfer and have limited attention to only five mutations. Furthermore we have assumed that selection acts only to increase resistance to cefotaxime, whereas microbes are exposed to a spatial and temporal diversity of antibiotic compounds in nature as well as in clinical settings (*1*). The implications of relaxing these assumptions are explored in the supporting online text.

However, this work was intended to answer a more fundamental evolutionary question: Given a set of point mutations known jointly to increase organismal fitness, how does Darwinian selection regard the many mutational trajectories available? The foregoing limitations notwithstanding, the implications of our study for this broader question are clear: When selection acts on TEM^{wt} to increase cefotaxime resistance, only a very small fraction of trajectories to *TEM** are likely to be realized, owing to sign epistasis mediated by intramolecular pleiotropic effects. Moreover, inasmuch as intramolecular pleiotropy (*11*, *25*) and concomitant sign epistasis are characteristic of many missense mutations (*25*), constraints on the selective choice of trajectories like those seen here are likely to apply to the evolution of other proteins. For example, application of our population genetic model to the fitness landscape between an engineered NADP- and the wild-type NAD-dependent forms of IMDH (*12*, *14*, *26*) reveals that at most 29% of all mutational trajectories are selectively accessible (supporting online text). Our conclusion is also consistent with results from prospective experimental evolution studies, in which replicate evolutionary realizations have been observed to follow largely identical mutational trajectories (*27*). However, the retrospective, combinatorial strategy employed here (*11*) substantially enriches our understanding of the process of molecular evolution because it enables us to characterize all mutational trajectories, including those with a vanishingly small probability of realization [which is otherwise impractical (*27*)]. This is important because it draws attention to the mechanistic basis of selective inaccessibility. It now appears that intramolecular interactions render many mutational trajectories selectively inaccessible, which implies that replaying the protein tape of life (*28*) might be surprisingly repetitive. It remains to be seen whether intermolecular interactions similarly constrain Darwinian evolution at larger scales of biological organization.

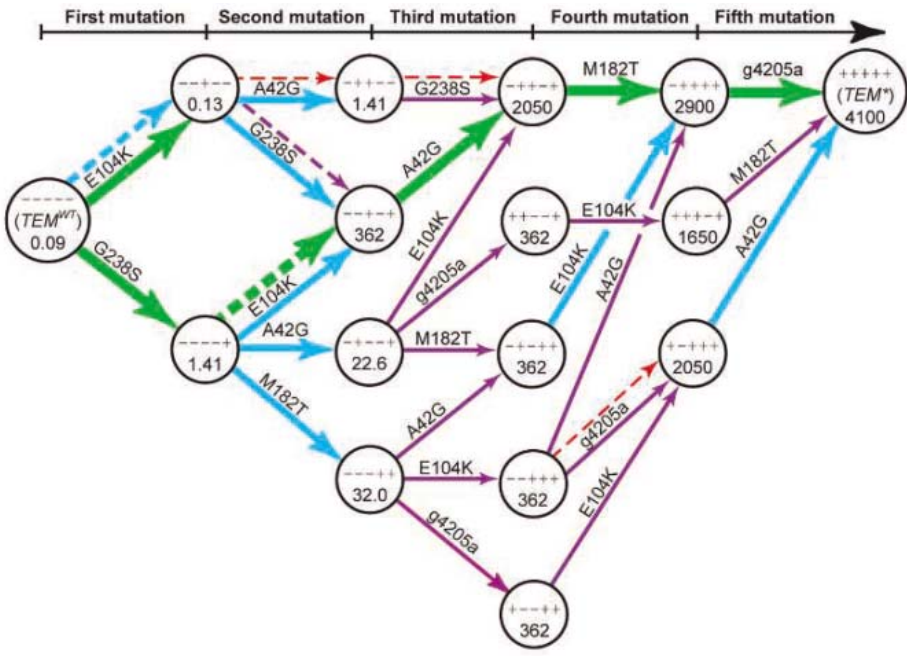

Fig. 2. Mutational composition of the 10 most probable trajectories from TEM^{wt} to *TEM**. Nodes represent alleles whose identities are given by a string of five + or – symbols corresponding (left to right) to the presence or absence of mutations g4205a, A42G, E104K, M182T, and G238S, respectively. Numbers indicate cefotaxime resistance (*12*) in μg/ml. Edges represent mutations, as labeled. The relative probability of each beneficial mutation is represented on a log scale by color and width of edges: green/wide, 0.316 to 1.0; purple/medium, 0.1 to 0.316; blue/narrow, 0.0316 to 0.1; and red/very narrow, less than 0.0316. Where two edges are shown between a pair of nodes, solid and broken edges correspond to probabilities under the equal and correlated fixation probability models, respectively. Elsewhere values differ between models by less than a factor of $\sqrt{10} = 0.316$.

Table 2. Summary of mutational effects on cefotaxime resistance.

Mutation	Number of TEM alleles on which mean mutational effect is			Mean† proportional increase
	Positive*	Negative*	Negligible	
g4205a	8‡	2‡	6	1.4
A42G	12	0	4	5.9
E104K	15	1	0	9.7
M182T	8‡	3‡	5	2.8
G238S	16	0	0	1.0×10^3

*Differences in mean MIC values are significant at $P < 0.05$. †Of MIC (*12*); geometric mean across all 16 alleles. ‡One of these comparisons loses significance after Bonferroni correction.

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29. M. Barlow and B. Hall introduced us to the TEM β-lactamase system and shared expertise and reagents with us. N. Shoresh suggested use of the likelihood framework and computed the critical values for the likelihood ratio test. D.M.W. and N.F.D. were supported by NSF under award DEB-0343598. M.A.D. is a Damon Runyon Fellow supported by the Damon Runyon Cancer Research Foundation (DRG-1861-05).

Supporting Online Material
www.sciencemag.org/cgi/content/full/312/5770/111/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 and S2
References and Notes

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Naïve and Memory CD4$^+$ T Cell Survival Controlled by Clonal Abundance

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Immunity to a plethora of microbes depends on a diverse repertoire of naïve lymphocytes and the production of long-lived memory cells. We present evidence here that low clonal abundance in a polyclonal repertoire favors the survival and activation of naïve CD4$^+$ T cells as well as the survival of their memory cell progeny. The inverse relation between clonal frequency and survival suggests that intraclonal competition could help maintain an optimally diverse repertoire of T cells and an optimal environment for the generation of long-lived memory cells.

Protective immunity against infectious disease depends on antigen-specific memory T cells that survive for many years following initial exposure to antigen (*1*). One major paradigm, based largely on studies of CD8$^+$ T cells, suggests that memory results from the conversion of naïve cells to long-lived memory cells that self-renew through the actions of the cytokine interleukin-15 (IL-15) (*1*). However, this mechanism may not apply to memory CD4$^+$ T cells because they are less dependent on IL-15 and may be derived from naïve precursors that are themselves long-lived (*1*). Furthermore, polyclonal virus–specific memory CD4$^+$ T cells have been seen to decline for almost a year after infection, indicating that not all memory CD4$^+$ T cells are stably maintained (*2*). Such discrepancies prompted us to study the stability of naïve and memory CD4$^+$ T cell populations.

To assess the in vivo survival of polyclonal naïve CD4$^+$ T cells, CD44^{low} CD4$^+$ cells from mice positive for the CD90.1 marker were tracked using antibodies to CD90.1 after adoptive transfer into congenic CD90.2$^+$ recipients (*3*). During the first 2 months, the transferred naïve CD4$^+$ T cells declined in the secondary lymphoid organs or blood of recipient mice with an estimated 50-day half-life (Fig. 1) (*4*), as reported by others (*5*, *6*). However, a longer observation period revealed an overall half-life estimate of 124 days, with 10% of the cells still remaining 1 year after transfer.

The survival time of transferred polyclonal naïve CD4$^+$ T cells was probably underestimated with this approach because of the loss of some CD44^{low} cells as a result of participation in immune responses to unknown foreign antigens. It was therefore necessary to test the survival of monoclonal naïve CD4$^+$ T cells that could not be unintentionally activated by foreign antigen. Monoclonal populations of CD4$^+$ T cells from ovalbumin peptide-I-A^d–specific DO11.10 (*7*) or flagellin peptide-I-A^b–specific SM1 (*8*) T cell antigen receptor (TCR) transgenic mice were used for this purpose. One million naïve DO11.10 or 4 × 10^6 SM1 CD4$^+$ T cells were transferred intravenously into histocompatible BALB/c or C57BL/6 mice, respectively, resulting in the seeding of about 10^5 cells in the spleen and lymph nodes (Fig. 2A and Fig. 3, A and B). The monoclonal cells then declined with half-lives of 12 and 7 days, respectively (Fig. 3A, triangles, and Fig. 3B, diamonds), revealing them to be short-lived as compared with their polyclonal counterparts. The poor survival of transferred naïve TCR transgenic cells was not related to rejection because potential sensitization of recipient mice by one injection of transgenic cells did not cause a second inoculum of transgenic cells to disappear more quickly (fig. S1).

The increased survival of polyclonal compared with monoclonal naïve CD4$^+$ T cells could have been related to clone size. The polyclonal repertoire of naïve T cells in a normal adult mouse is composed of about 2 × 10^6 unique clones of 50 cells each (*9*, *10*). Thus, 10^5 transferred monoclonal cells represents a clone size of ~2000 times that of the typical naïve clonal population. However, the conventional approach of sampling a small fraction of secondary lymphoid tissue to enumerate adoptively transferred T cells was inadequate to detect the 100 cells seeded by a 1000-cell transfer (Fig. 2B) because the background was too high (Fig. 2C).

The conventional method was improved using a magnetic bead–based enrichment step that concentrated all of the transferred cells from the spleen and lymph nodes of individual mice (compare Fig. 2, A and D) into a small volume and reduced the number of contaminating recipient cells by a factor of several hundred. This enrichment method enabled detection of

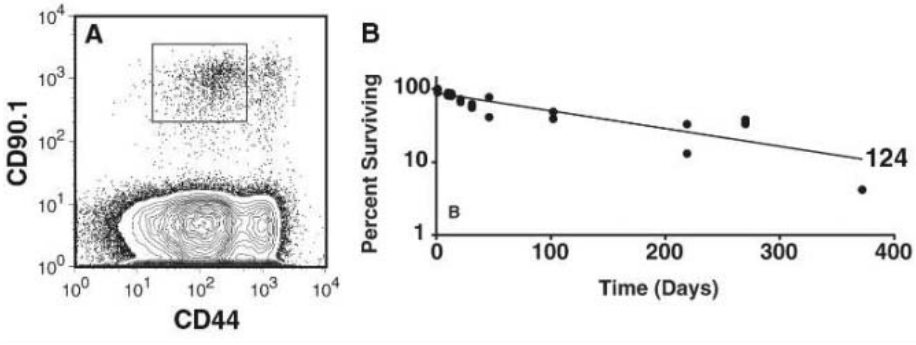

Fig. 1. Polyclonal naïve CD4$^+$ T cells persist in normal recipients for more than 1 year. Two million polyclonal CD90.1$^+$ CD4$^+$ T cells were injected into CD90.2$^+$ C57BL/6 mice. (**A**) The naïve transferred T cells were then identified as CD4$^+$, B220$^-$ (not shown), CD90.1$^+$, CD44^{low} cells. (**B**) The percentage of these cells remaining in each recipient (pooled from two independent experiments) over time, fit to an exponential decay curve. The estimated half-life of the population (in days) is shown.

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about 100 cells seeded by a 1000-cell transfer (Fig. 2E) and reduced the background level to fewer than 20 cells (Fig. 2F). The background was reduced to 0 by excluding cells that bound antibody to CD4 nonspecifically (Fig. 2G) and by transferring cells that expressed CD90.1 as an additional marker. This low background rate allowed detection of only 10 transferred cells (compare Fig. 2, H and I).

Using the enrichment method described here, we found that 10 or 80 naïve DO11.10 cells per mouse survived with half-lives of 50 and 104 days, respectively. Both half-lives represent a significant increase over the 12-day half-life of 10^5 cells ($P < 0.01$) (Fig. 3A) and were similar to the survival time seen for polyclonal naïve CD4$^+$ T cells. Likewise, 50 or 200 naïve SM1 cells per mouse survived with half-lives of greater than 100 days, which again were significantly increased compared with the 7-day half-life of 3×10^5 cells ($P < 0.01$) (Fig. 3B). Not only were naïve monoclonal T cells longer lived at low frequency, but they also underwent transient cell division reminiscent of homeostatic proliferation (Fig. 4), which is typically observed in lymphopenic hosts (*1*). Therefore, naïve monoclonal CD4$^+$ T cells proliferated more and survived longer when present at physiologically appropriate numbers than at a much higher frequency.

Clonal abundance was also an important factor in the activation of naïve cells by foreign antigen. Starting from 10^5 per recipient, naïve DO11.10 cells divided fewer than eight times (fig. S2) and increased by a factor of 20 to a peak 3 days after intravenous injection of the relevant foreign antigen, ovalbumin peptide (Fig. 3C, triangles). The cells then declined rapidly to 10^5 per recipient on day 9, converted to the CD45RB^{low} phenotype (*11*), and survived (Fig. 3C, triangles) with a short 11-day half-life that was not significantly different ($P = 0.29$) than that of 10^5 naïve DO11.10 cells (Fig. 3A, triangles). In contrast, an initial cohort of about 100 naïve DO11.10 cells underwent more efficient activation, dividing at least eight times (fig. S2) and increasing by a factor of more than 200 to a later peak on day 8 (Fig. 3C, diamonds). The cells then declined rapidly until day 21 to 1500 cells and survived during the memory phase with a half-life of 46 days (Fig. 3C, diamonds). In a similar experiment starting from 500 naïve DO11.10 cells, 5000 cells entered the memory phase and survived with a half-life of 40 days (Fig. 3C, circles). The 46-day and 40-day half-lives of 1500 and 5000 memory cells, respectively, were each significantly longer than the 11-day half-life of 10^5 memory cells ($P < 0.01$) (Fig. 3C, triangles). Therefore, low naïve CD4$^+$ T cell clonal abundance accentuated antigen-driven proliferation and resulted in the generation of memory cells with increased survival.

Notably, monoclonal memory CD4$^+$ T cells that were generated from a large number of naïve cells and declined in their original recipients with a 10-day half-life survived with a significantly longer half-life of 28 days after transfer into new naïve recipients at a level of 40 cells per mouse ($P < 0.01$) (Fig. 3D). Thus, like naïve cells, memory cells benefited from a low clonal abundance. However, clonal abundance was not the only determinant of survival, because memory cells generated from a large number of naïve cells did not survive as well at low frequency (Fig. 3D, open triangles) as naïve cells at the same frequency (Fig. 3B, squares) ($P = 0.01$). Therefore, the greater potency of stimulation that rare naïve cells experience during the primary response likely promotes the formation of inherently long-lived memory cells. This possibility is supported by the recent finding that low naïve cell frequency is important for the formation of stable effector memory CD8$^+$ T cells (*12*).

Previous studies showed that CD4$^+$ T cell survival in lymphopenic hosts is not controlled by the TCR or MHC II (major histocompatibility complex II) molecules (*5*, *13*), perhaps because of compensation by a cytokine such as IL-7 (*14*). In contrast, our results suggest that monoclonal CD4$^+$ T cells in full hosts compete for a clone-specific survival signal that is most easily explained by TCR recognition of a specific and limiting self peptide-MHC II ligand. This model is supported by the finding that induced ablation of the TCR or MHC II shortens the life span of polyclonal CD4$^+$ T cells (*15*–*17*). In addition, the observations that monoclonal CD4$^+$ T cells do not compete with other clones (*18*–*21*), and that most polyclonal CD4$^+$ T cells do not survive well when transferred into hosts containing a single self peptide-MHC II ligand (*22*), suggest that the relevant TCR ligands are clone specific. Intraclonal competition might also explain the finding that virus antigen–specific memory CD4$^+$ T cells decline after infection is cleared and then stabilize as they become less abundant (*2*). The tendency identified here for

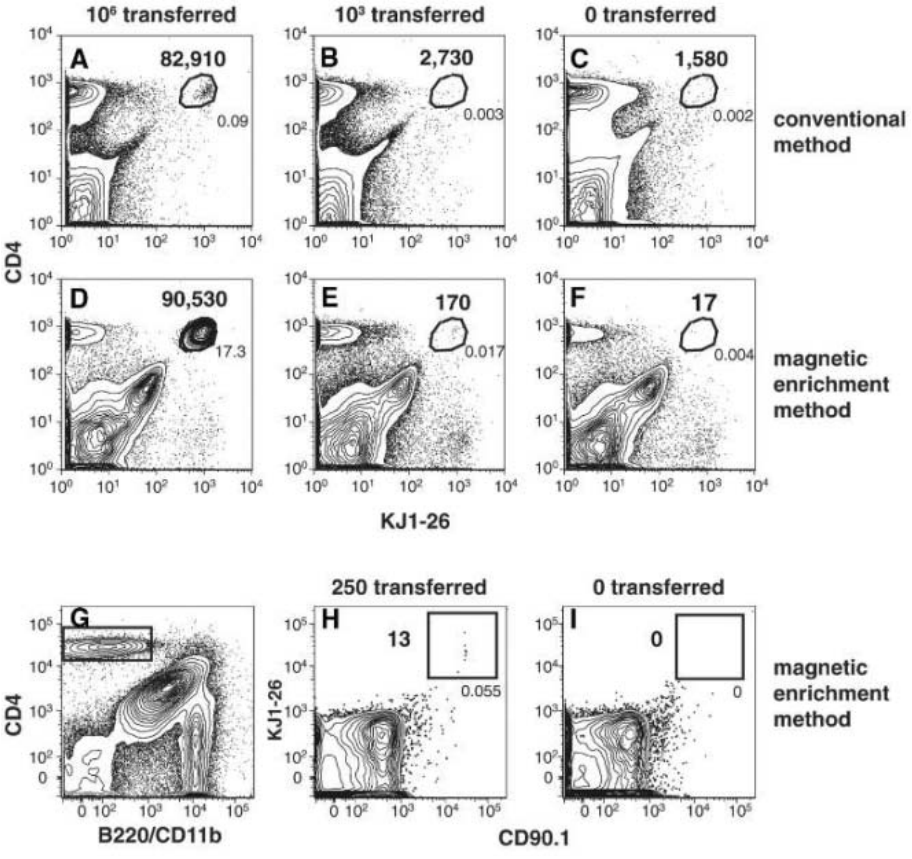

Fig. 2. Rare monoclonal T cells can be detected after transfer by an enrichment method. CD4$^+$, KJ1-26$^+$ cells detected by the conventional flow cytometry method (**A** to **C**) (*24*) or the enrichment method (**D** to **F**) in the spleen and mesenteric lymph nodes of BALB/c mice that received 10^6 [(A) and (D)], 10^3 [(B) and (E)], or no [(C) and (F)] DO11.10 T cells. The percentage and total number of DO11.10 cells detected in each sample are shown on each plot to the right and above the CD4$^+$, KJ1-26$^+$ gate, respectively. To test the sensitivity of the system, 250 CD90.1$^+$ DO11.10 T cells were transferred into CD90.2$^+$ recipients and detected as B220$^-$, CD11b$^-$, CD4$^+$ (**G**), KJ1-26$^+$, CD90.1$^+$ (**H**) cells. Inclusion of the CD90.1 marker and exclusion of B220$^+$ and CD11b$^+$ cells reduced the background to zero in four mice that did not receive DO11.10 cells (**I**).

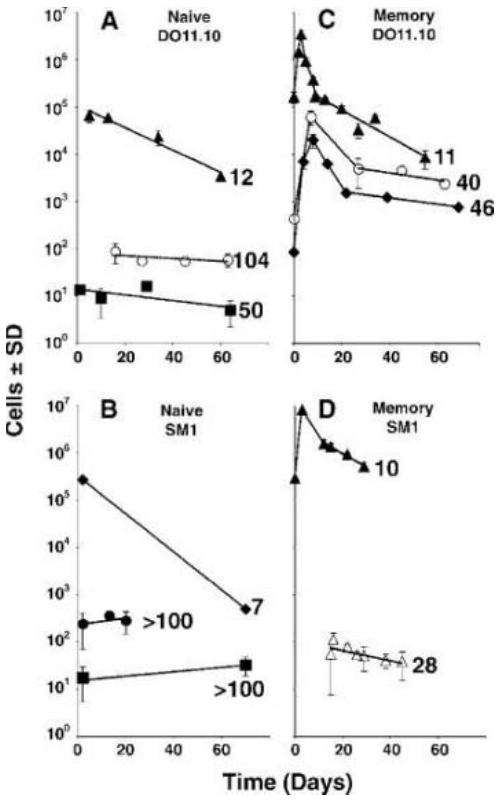

Fig. 3. Monoclonal CD4$^+$ T cells quickly decline at high frequency but persist at low frequency. (**A**) Naïve DO11.10 cells remaining in the spleen and lymph nodes of recipient BALB/c mice after initial parking of 10^5 (triangles), 80 (circles), or 13 (squares) cells. (**B**) Naïve SM1 cells remaining in C57BL/6 mice after seeding of 3×10^5 (diamonds), 200 (circles), or 50 (squares) cells. (**C**) DO11.10 cells in BALB/c mice after initial seeding of 10^5 (triangles), 500 (circles), or 80 (diamonds) cells and intravenous injection of ovalbumin peptide plus lipopolysaccharide (LPS) on day 0. (**D**) SM1 cells in C57BL/6 mice after initial seeding of 3×10^5 cells and intravenous injection of FliC peptide plus LPS (filled triangles), or memory SM1 cells that were generated in C57BL/6 recipients from 3×10^5 naïve cells by intravenous injection of FliC peptide plus LPS and then seeded into new naïve recipients on day 12 at 40 cells per mouse (open triangles, pooled from two experiments). All of the individual points shown are greater than the limit of detection defined as 1 SD above the average number of events detected in at least four untransferred mice assayed on different days over the time course. The individual values at each time point for the naïve groups (A and B) and the values for the memory groups at day 10 [(C) and (D), filled triangles)] or day 20 [(C), open circle and filled diamonds)] were fit to exponential curves. The estimated half-lives (in days) are shown near each curve.

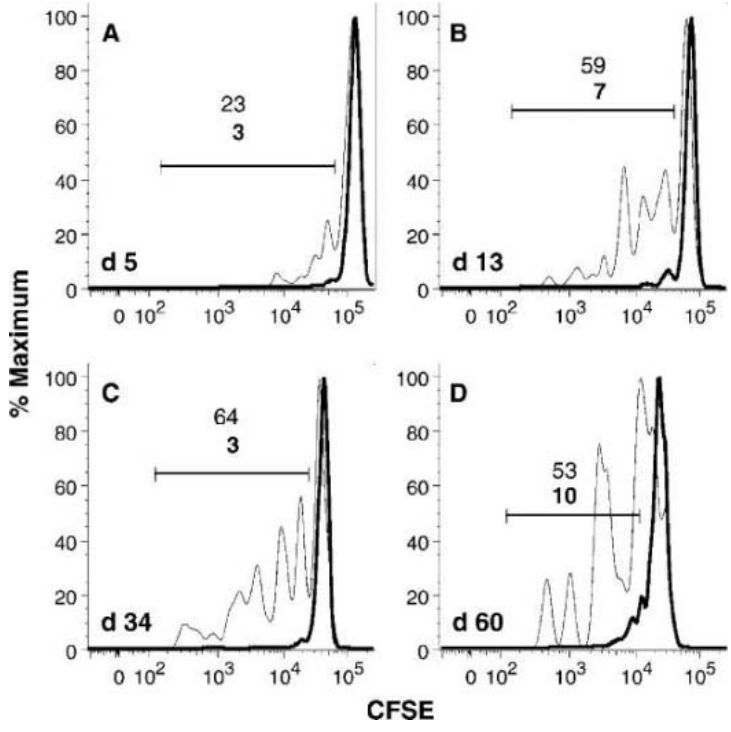

Fig. 4. Naïve CD4$^+$ T cells undergo a short burst of cell division after transfer at low frequency, but not high frequency. Carboxyfluorescein diacetate succinimidyl ester (CFSE) histograms for naïve DO11.10 T cells (identified as B220$^-$, CD11b$^-$, CFSE$^+$, CD4$^+$, KJ1-26$^+$ cells) initially seeded at 10^5 (thick line) or 500 (thin line) cells in the spleen and lymph nodes of BALB/c recipients 5 (**A**), 13 (**B**), 34 (**C**), or 60 (**D**) days after transfer. The percentages of cells with one or more cell divisions in the high (bold text) or low (regular text) transfer groups are indicated.

individual clones to be maintained in small numbers may explain how millions of different naïve clones can coexist stably and how single memory cell populations are prevented from squeezing out others (*23*).

Together these results suggest that the stability of individual CD4$^+$ T cell clones is due to their independent regulation in separate niches. This permits the simultaneous long-term survival of millions of unique clones, thereby maximizing the chance that a few will be specific for a given pathogen. In the event of infection, the low abundance of the relevant clones will also ensure minimal competition for foreign antigen, maximizing activation and the formation of long-lived memory cells.

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Supporting Online Material

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Losartan, an AT1 Antagonist, Prevents Aortic Aneurysm in a Mouse Model of Marfan Syndrome

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Aortic aneurysm and dissection are manifestations of Marfan syndrome (MFS), a disorder caused by mutations in the gene that encodes fibrillin-1. Selected manifestations of MFS reflect excessive signaling by the transforming growth factor–β (TGF-β) family of cytokines. We show that aortic aneurysm in a mouse model of MFS is associated with increased TGF-β signaling and can be prevented by TGF-β antagonists such as TGF-β–neutralizing antibody or the angiotensin II type 1 receptor (AT1) blocker, losartan. AT1 antagonism also partially reversed noncardiovascular manifestations of MFS, including impaired alveolar septation. These data suggest that losartan, a drug already in clinical use for hypertension, merits investigation as a therapeutic strategy for patients with MFS and has the potential to prevent the major life-threatening manifestation of this disorder.

MFS is a systemic disorder of connective tissue caused by mutations in *FBN1*, the gene encoding fibrillin-1 (*1*). As a principal component of the extracellular matrix microfibril (*2*, *3*), fibrillin-1 was initially thought to play primarily a structural role in connective tissue. Several lines of evidence support an additional role as a regulator of the cytokine TGF-β (*4*, *5*). Mice homozygous for a hypomorphic *Fbn1* allele have impaired pulmonary alveolar septation associated with increased TGF-β signaling that can be prevented by perinatal administration of a polyclonal TGF-β neutralizing antibody (NAb) (*5*). Similarly, myxomatous thickening of the cardiac atrioventricular valves in mice harboring a *Fbn1* missense mutation is attenuated by perinatal systemic administration of TGF-β NAb (*6*).

We sought to determine the role of TGF-β in MFS-associated aortic aneurysm, which is the major life-threatening manifestation of this condition. We studied mice heterozygous for an *Fbn1* allele encoding a cysteine substitution, $Cys^{1039} \rightarrow Gly$ (C1039G), in an epidermal growth factor–like domain of fibrillin-1 ($Fbn1^{C1039G/+}$) (*6*–*8*), which is the most common class of mutation causing MFS. The aortic root in $Fbn1^{C1039G/+}$ mice undergoes progressive dilatation, evident as early as 2 weeks of age. By 7 weeks of age, the aortic root in the mutant mice is larger than that in wild-type mice (1.82 ± 0.14 mm versus 1.59 ± 0.11 mm, respectively; $P < 0.05$). This size difference becomes more pronounced with age (aortic root at 8 months, 2.47 ± 0.33 mm versus 1.82 ± 0.11 mm; $P < 0.0001$).

Histologic analysis of 14-week-old $Fbn1^{C1039G/+}$ mice revealed aberrant thickening of the aortic media with fragmentation and disarray of elastic fibers (fig. S1). In addition, $Fbn1^{C1039G/+}$ mice showed increased collagen deposition, which is an indirect marker of increased TGF-β signaling (fig. S1) (*9*, *10*). Phosphorylation and subsequent nuclear translocation of Smad2 (pSmad2), which are induced by TGF-β signaling (*11*), are markedly increased in the aortic media of $Fbn1^{C1039G/+}$ mice relative to wild-type mice (fig. S1). Similar changes have been observed in aortic samples derived from patients with MFS (*12*).

To investigate whether excessive TGF-β signaling plays a causal role in progressive aortic root enlargement, we treated mice postnatally with TGF-β NAb after the establishment of aortic root aneurysm (Fig. 1). Treatment by intraperitoneal injection was begun at 7 weeks of age and continued for 8 weeks. The $Fbn1^{C1039G/+}$ mice received low-dose TGF-β NAb (1 mg/kg body weight; Fig. 1, C and G), high-dose TGF-β NAb (10 mg/kg; Fig. 1, D and H), or placebo (10 mg/kg rabbit IgG; Fig. 1, B and F) every 2 weeks. Histologic analyses revealed reduced elastic fiber fragmentation and reduced TGF-β signaling in the aortic media of TGF-β Nab–treated mice relative to the placebo group (Fig. 1, A to H). In humans with MFS, the diameter and rate of enlargement of the aortic root are directly proportional to the risk of life-threatening aortic dissection (*13*). Echocardiograms revealed that the aortic root diameter at baseline in the wild-type mice (1.57 ± 0.05 mm) was smaller than that in the three $Fbn1^{C1039G/+}$ treatment arms [placebo, 1.75 ± 0.15 mm; NAb (10 mg/kg), 1.80 ± 0.11 mm; NAb (1 mg/kg), 1.86 ± 0.15 mm; $P < 0.0001$ for each treatment arm relative to wild type]. There was no difference in the growth rate of the aortic root, as assessed by echocardiograms performed after 8 weeks of treatment, between wild-type mice and either of the TGF-β NAb treatment groups ($P = 0.11$). In contrast, the aortic root growth rate in the placebo-treated mice was greater than that in either wild-type ($P < 0.0001$) or NAb-treated mice ($P < 0.03$, Fig. 1I). After 8 weeks, aortic wall thickness in NAb-treated $Fbn1^{C1039G/+}$ mice was indistinguishable from that in untreated wild-type mice ($P = 0.91$) and less than that in the placebo group ($P < 0.01$, Fig. 1J). Aortic wall architecture was disrupted in $Fbn1^{C1039G/+}$ mice relative to wild-type mice ($P < 0.0001$) but improved in mutant mice treated with NAb ($P < 0.001$, Fig. 1K). These data show that excessive TGF-β signaling contributes to the formation of aortic aneurysm in a mouse model of MFS, and that TGF-β antagonism represents a productive treatment strategy.

We became interested in losartan, an angiotensin II type 1 receptor (AT1) antagonist, not only because it lowers blood pressure—a desirable effect in patients with aortic aneurysm—but also because it leads to antagonism of TGF-β in animal models of chronic renal insufficiency and cardiomyopathy (*14*, *15*). Using a prenatal administration protocol in our mouse model, we compared the efficacy of losartan to that of propranolol, which is representative of β-adrenergic blocking agents widely used in patients with MFS to slow the rate of aortic growth (*16*). The doses of losartan and propranolol were titrated to achieve comparable hemodynamic effects in vivo, including a 15 to 20% decrease in heart rate and a 10 to 20% decrease in blood pressure in both groups.

Pregnant $Fbn1^{C1039G/+}$ mice received losartan (0.6 g/liter), propranolol (0.5 g/liter), or placebo in their drinking water, beginning at 2 weeks of gestation. Treatment of the mothers continued throughout lactation and was maintained in the pups after weaning. Mice were killed at 10 months of age. Elastic fiber fragmentation was observed in both placebo- and propranolol-treated mice, but not in losartan-treated mice (Fig. 2, A to D). The average aortic wall thickness for untreated wild-type animals was smaller than that in placebo-treated $Fbn1^{C1039G/+}$ mice ($P < 0.0001$) but was indistinguishable from that in losartan-treated $Fbn1^{C1039G/+}$ mice ($P = 0.24$, Fig. 2E). Aortic wall thickness in the propranolol-treated mice was indistinguishable from that in the placebo

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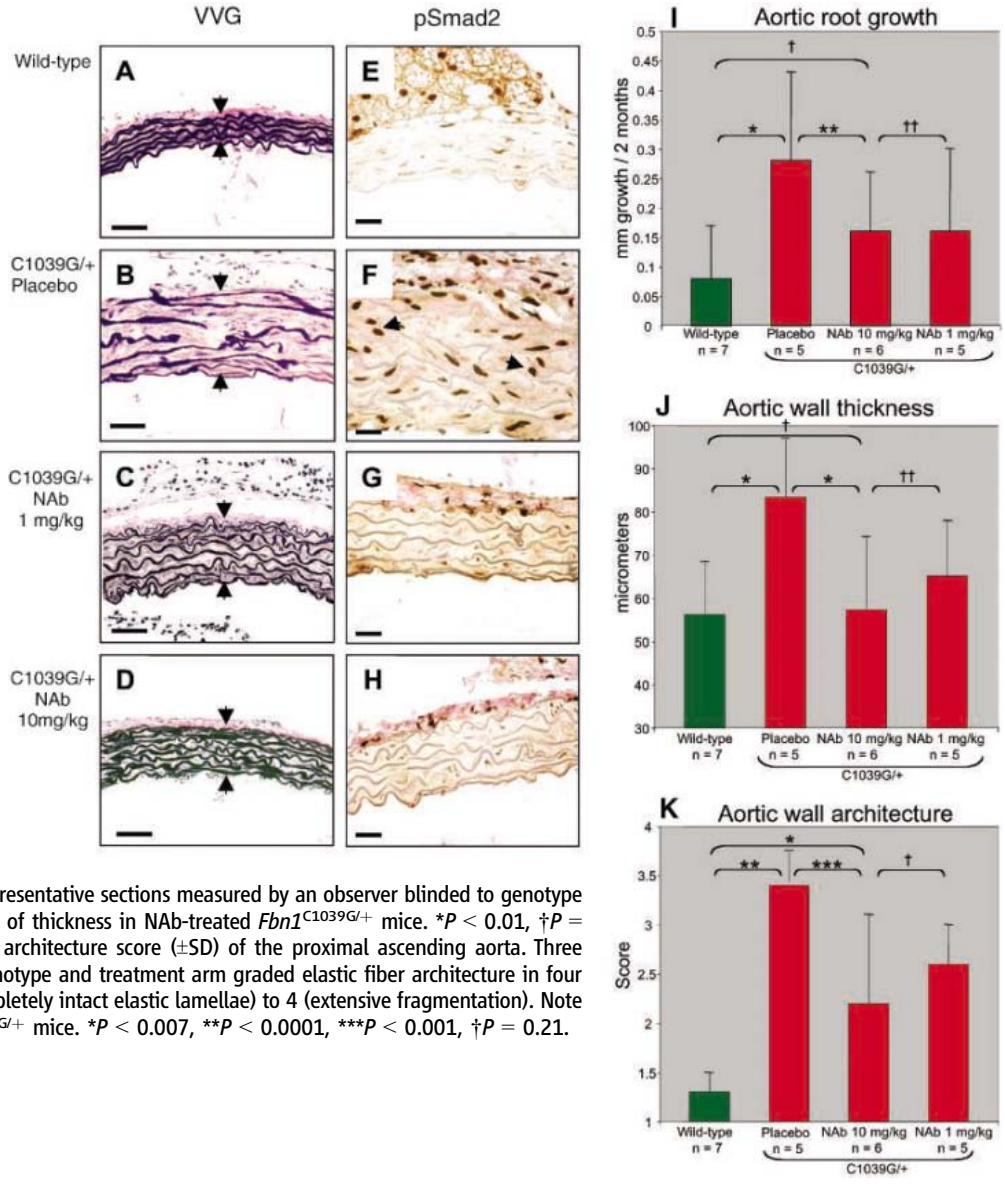

Fig. 1. Postnatal treatment with TGF-β NAb. (**A** to **H**) Characterization of the ascending aorta in untreated wild-type mice [(A) and (E)] and *Fbn1*$^{C1039G/+}$ mice treated with placebo [(B) and (F)], 1 mg/kg TGF-β NAb [(C) and (G)], and 10 mg/kg TGF-β NAb [(D) and (H)]. In (A) to (D), Verhoeff's–van Gieson (VVG) stain reveals diffuse disruption of elastic fiber architecture and thickening of the aortic media (delineated by arrows) in placebo-treated *Fbn1*$^{C1039G/+}$ mice (B) relative to the normal elastic fiber architecture observed in wild-type mice (A). Improvement in both parameters is seen in NAb-treated *Fbn1*$^{C1039G/+}$ mice [(C) and (D)]. Scale bars, 40 μm. In (E) to (H), immunohistochemistry (IH) reveals nuclear pSmad2, a marker for TGF-β signaling (arrows indicate representative positive nuclei). Increased pSmad2 is observed in the placebo-treated *Fbn1*$^{C1039G/+}$ mice (F) relative to wild-type mice (E). Normalized pSmad2 staining is observed in the NAb-treated *Fbn1*$^{C1039G/+}$ mice [(G) and (H)]. Scale bars, 50 μm. (**I**) Average aortic root growth (±SD) measured by echocardiogram over the 2-month treatment period. Note the reduced rate of growth in the NAb-treated mice relative to the placebo-treated *Fbn1*$^{C1039G/+}$ mice. **P* < 0.0001, ***P* < 0.03, †*P* = 0.11, ††*P* = 1.0. (**J**) Average thickness (±SD) of the proximal ascending aortic media of four representative sections measured by an observer blinded to genotype and treatment arm. Note full normalization of thickness in NAb-treated *Fbn1*$^{C1039G/+}$ mice. **P* < 0.01, †*P* = 0.91, ††*P* = 0.38. (**K**) Average aortic wall architecture score (±SD) of the proximal ascending aorta. Three separate observers who were blinded to genotype and treatment arm graded elastic fiber architecture in four representative areas on a scale from 1 (completely intact elastic lamellae) to 4 (extensive fragmentation). Note the improvement in NAb-treated *Fbn1*$^{C1039G/+}$ mice. **P* < 0.007, ***P* < 0.0001, ****P* < 0.001, †*P* = 0.21.

group (*P* = 0.19). Likewise, aortic wall architecture was normalized in losartan-treated *Fbn1*$^{C1039G/+}$ animals relative to the placebo group (*P* < 0.0001) but was not influenced by propranolol (*P* = 0.16, Fig. 2F). There was marked aortic dilatation in the placebo- and propranolol-treated mutant mice, whereas the losartan-treated mutant mice were indistinguishable from wild-type littermates (fig. S2).

Because MFS is typically diagnosed after birth and because the use of AT1 antagonists is contraindicated during pregnancy (*17*), we investigated whether losartan could attenuate or prevent abnormal aortic root growth if treatment were initiated postnatally, after the establishment of aortic aneurysms. At 7 weeks of age, after echocardiographic documentation of aneurysm (fig. S3), *Fbn1*$^{C1039G/+}$ mice received placebo, propranolol (0.5 g/liter), or losartan (0.6 g/liter) in their drinking water. Baseline echocardiograms revealed no differences in aortic root size between any of the treatment groups for *Fbn1*$^{C1039G/+}$ mice (placebo 1.83 ± 0.11 mm, propranolol 1.92 ± 0.27 mm, losartan 1.84 ± 0.08 mm, respectively; *P* = 0.5). However, before treatment, the aortic diameter in *Fbn1*$^{C1039G/+}$ mice was always greater than in untreated wild-type mice (1.59 ± 0.11 mm; *P* < 0.002) (fig. S3).

Three independent aortic root measurements were obtained for each mouse every 2 months during the 6 months of treatment. Mice were killed at 8 months of age. In contrast to propranolol or placebo, losartan treatment prevented elastic fiber fragmentation (Fig. 3, A to D) and blunted TGF-β signaling in the aortic media, as evidenced by reduced nuclear accumulation of pSmad2 (Fig. 3, E to H). The aortic root growth rate over this period was less in the wild-type mice than in the placebo-treated *Fbn1*$^{C1039G/+}$ mice (*P* < 0.0001, Fig. 3I). Although the propranolol-treated *Fbn1*$^{C1039G/+}$ mice did show a slower rate of aortic root growth than did placebo-treated animals (*P* < 0.001), this growth rate remained greater than that in untreated wild-type mice (*P* < 0.04). In contrast, the aortic root growth rate in losartan-treated *Fbn1*$^{C1039G/+}$ mice was indistinguishable from that in the wild-type group (*P* = 0.55, Fig. 3I). Furthermore, the absolute diameter of the aortic root at the end of treatment was similar in the losartan-treated *Fbn1*$^{C1039G/+}$ mice and untreated wild-type littermates (*P* = 0.32; fig. S3). Propranolol had no discernable effect on either aortic wall thickness or elastic fiber architecture when compared to placebo; hence, its beneficial effect is limited to slowing the rate of growth of the aortic root. In contrast, losartan-treated *Fbn1*$^{C1039G/+}$ mice showed improvement in all three parameters compared to placebo-treated mice, with full normalization relative to wild-type mice (Fig. 3, I to K). We conclude that

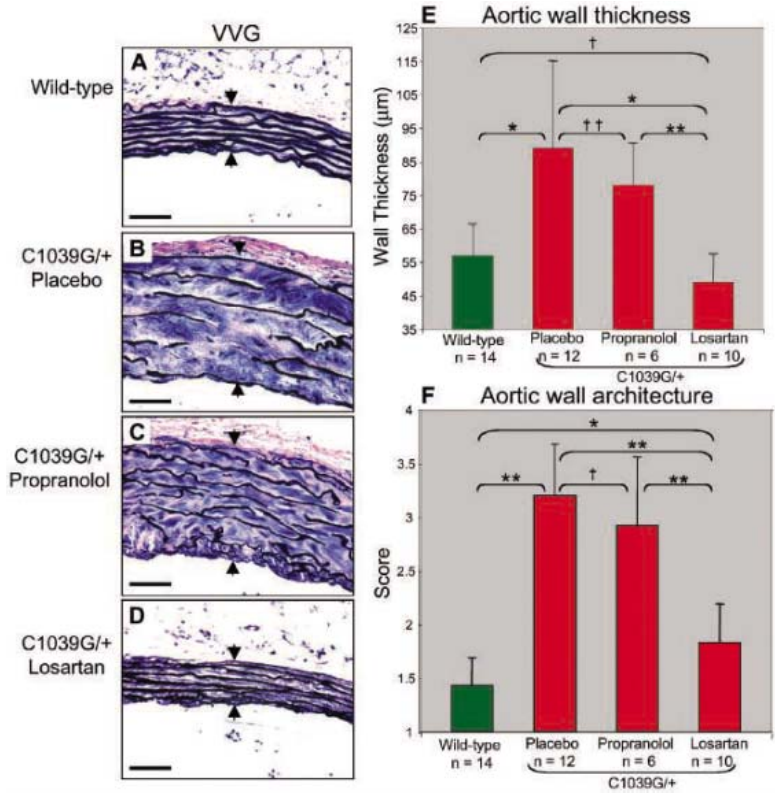

Fig. 2. Prenatal treatment with losartan and propranolol. (**A** to **D**) VVG staining highlights intact elastic fiber architecture and normal ascending aortic wall thickness (arrows) in wild-type mice (A) and losartan-treated *Fbn1*$^{C1039G/+}$ mice (D). Marked elastic fiber disruption and wall thickening is apparent in the placebo- and propranolol-treated *Fbn1*$^{C1039G/+}$ mice [(B) and (C).] Scale bars, 40 μm. (**E**) Average aortic wall thickness (±SD) after 10 months of treatment. Note full normalization of wall thickness in losartan-treated *Fbn1*$^{C1039G/+}$ mice relative to mice that received placebo or propranolol treatment. *$P < 0.0001$, **$P < 0.002$, †$P = 0.24$, ††$P = 0.19$. (**F**) Average aortic wall architecture score (±SD) after treatment. Note the improvement in losartan-treated *Fbn1*$^{C1039G/+}$ mice. *$P < 0.02$, **$P < 0.0001$, †$P = 0.16$.

β-adrenergic blockade with propranolol diminishes aortic growth rate in this model of MFS but does not prevent progressive deterioration of aortic wall architecture or ongoing abnormal aortic dilatation. In contrast, AT1 blockade with losartan appears to achieve full correction of the phenotypic abnormalities in the aortic wall of *Fbn1*$^{C1039G/+}$ mice.

In a previous study, we showed that a different strain of mice homozygous for hypomorphic *Fbn1* alleles showed widening of the distal airspace due to failure of alveolar septation (*5*). This abnormality correlated with increased TGF-β signaling and was prevented by prenatal administration of TGF-β NAb (*5*). To determine whether losartan can improve this lung phenotype when administered postnatally—a matter of specific relevance to patients with MFS—we treated *Fbn1*$^{C1039G/+}$ mice with losartan beginning at 7 weeks of age. After 6 months of treatment, placebo-treated *Fbn1*$^{C1039G/+}$ mice showed widening of the distal airspace due to impaired alveolar septation (mean linear intercept 84.3 ± 15 μm) relative to wild-type placebo-treated mice (mean linear intercept 41.3 ± 5 μm, $P < 0.001$; Fig. 4). Losartan-treated *Fbn1*$^{C1039G/+}$ mice showed a reduction in distal airspace caliber relative to placebo-treated *Fbn1*$^{C1039G/+}$ animals (mean linear intercept 53.9 ± 12 μm, $P < 0.001$; Fig. 4).

AT1 antagonism might achieve superior protection over β-adrenergic blocking agents by virtue of increased blunting of the hemodynamic stress that is imposed on a structurally deficient aortic wall, as opposed to a mechanism relevant to TGF-β signaling or other molecular pathogenetic events. Four lines of evidence argue against this hypothesis. First, the doses of losartan and propranolol were titrated to achieve comparable hemodynamic effects. Second, isolated antagonism of TGF-β signaling with NAb provides similar protection. Third, analysis of pSmad2 nuclear staining revealed that losartan antagonizes TGF-β signaling in the aortic wall of *Fbn1*$^{C1039G/+}$ mice, an event seen in NAb-treated mice but not in propranolol-treated mice (Fig. 3, G and H). Fourth, we demonstrate here that losartan can improve disease manifestations in the lungs (Fig. 4), an event that cannot plausibly relate to improved hemodynamics.

The mechanism by which AT1 blockade antagonizes TGF-β signaling remains to be fully elucidated. Signaling through the AT1 receptor increases the expression of TGF-β ligands and receptors and also induces the expression of thrombospondin-1, a potent activator of TGF-β (*18*–*21*). In the vessel wall, AT1 signaling stimulates proliferation of vascular smooth muscle cells (VSMCs) and vessel wall fibrosis (*22*), although this may be context-dependent. In avian systems, neural crest– and mesoderm-derived VSMCs (N- and M-VSMCs, respectively) respond differently to TGF-β1, with cellular proliferation and fibrosis seen in the former and growth inhibition seen in the latter (*23*, *24*). This differential response may explain the particular predisposition of the root of the aorta—a vascular segment enriched for N-VSMCs—to undergo dilatation and dissection in MFS. The pulmonary artery root is also enriched for N-VSMCs and routinely shows dilatation in MFS despite the reduced pressure in the pulmonary circulation (*25*).

Given that signaling through the angiotensin II type 2 receptor (AT2) antagonizes many of the effects that are promoted by AT1 signaling (*26*), specific AT1 antagonism may be preferable to the dual AT1/AT2 blockade achieved with angiotensin converting enzyme (ACE) inhibitors. Consistent with this hypothesis, Daugherty and colleagues found that the formation of angiotensin II–induced abdominal aortic aneurysms could be prevented in apoE$^{-/-}$ mice by treatment with AT1 antagonists but was accelerated in both frequency and severity upon treatment with a selective AT2 blocker (*27*). Nagashima and colleagues observed increased apoptosis in vessel wall explants and cultured VSMCs from individuals with MFS, and they showed that AT2 but not AT1 blockade reduced this effect (*28*, *29*). These samples were derived from aneurysms in the 7- to 9-cm range, far beyond the current threshold for aortic root surgery. In our mouse model, we have not detected enhanced apoptosis in early and intermediate stages of aortic aneurysm (fig. S4). Angiotensin II also stimulates Smad2-dependent signaling and fibrosis in VSMCs in a TGF-β–independent manner, and this effect can be prevented by selective AT1 blockade (*30*). Thus, although TGF-β ligand-dependent signaling appears critical to the pathogenesis of aortic aneurysm in MFS, antagonism of a parallel pSmad2-mediated signaling cascade may contribute to the protection afforded by losartan.

The demonstration of excess TGF-β signaling in the aortic wall of patients with other aortic aneurysm syndromes, including Loeys-Dietz syndrome (caused by mutations in *TGFBR1* or *TGFBR2*) and arterial tortuosity syndrome (caused by mutations in *GLUT10*), suggests that losartan may be of broad relevance in the treatment of human vasculopathies (*12*, *31*).

Losartan is currently in widespread clinical use for treatment of hypertension and prevention

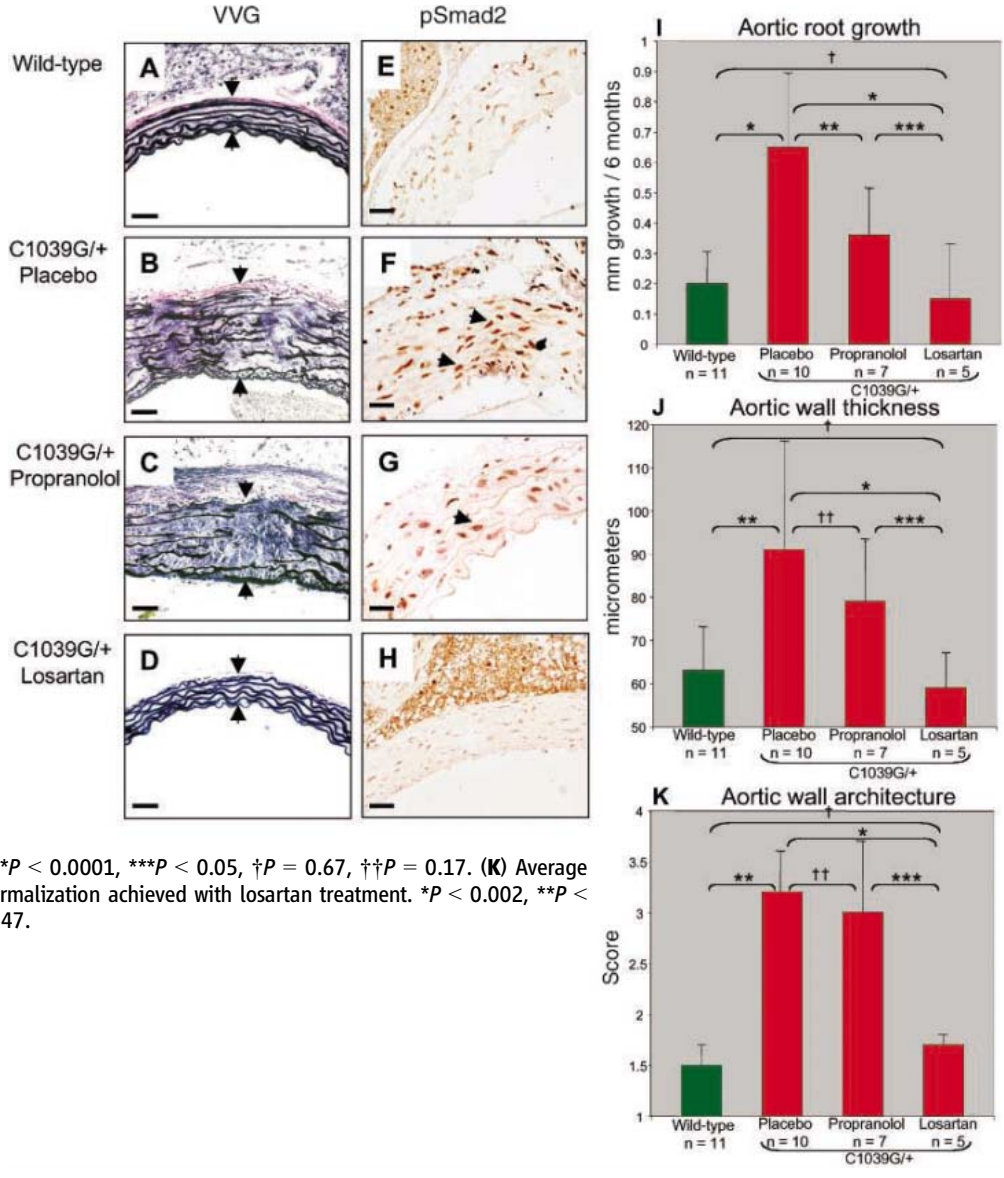

Fig. 3. Postnatal treatment with losartan and propranolol. (**A** to **H**) VVG staining [(A) to (D)] and pSmad2 immunostaining [(E) to (H)] of aortic wall. Elastic lamellae are intact and aortic media is of normal thickness in the wild-type (A) and losartan-treated *Fbn1*$^{C1039G/+}$ mice (D). Placebo- and propranolol-treated *Fbn1*$^{C1039G/+}$ mice [(B) and (C)] have diffuse fragmentation of elastic fibers and thickening of the aortic media (arrows). Scale bars, 50 μm. Nuclear pSmad2 staining is decreased in the aortic media of wild-type (E) and losartan-treated *Fbn1*$^{C1039G/+}$ mice (H). Marked increase in nuclear staining for pSmad2 (representative positive cells denoted by arrowheads) is seen in the *Fbn1*$^{C1039G/+}$ mice treated with placebo (F) and propranolol (G). Scale bars, 40 μm. (**I**) Average aortic root growth (±SD) during the 6 months of treatment. Note that aortic root growth in *Fbn1*$^{C1039G/+}$ mice treated with propranolol is less than that with placebo, yet remains greater than that seen in wild-type mice. Losartan treatment normalizes growth rate. $*P < 0.0001$, $**P < 0.001$, $***P < 0.02$, $\dagger P = 0.55$. (**J**) Average aortic wall thickness (±SD). Aortic wall thickness in losartan-treated *Fbn1*$^{C1039G/+}$ mice is reduced relative to placebo- and propranolol-treated mice and is indistinguishable from that seen in wild-type mice. $*P < 0.002$, $**P < 0.0001$, $***P < 0.05$, $\dagger P = 0.67$, $\dagger\dagger P = 0.17$. (**K**) Average aortic wall architecture (±SD). Note full normalization achieved with losartan treatment. $*P < 0.002$, $**P < 0.0001$, $***P < 0.05$, $\dagger P = 0.20$, $\dagger\dagger P = 0.47$.

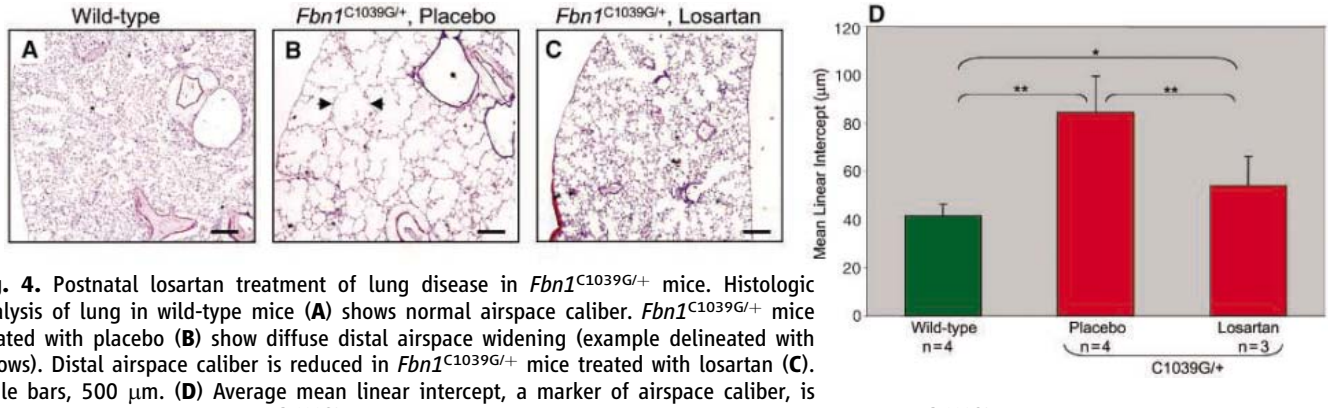

Fig. 4. Postnatal losartan treatment of lung disease in *Fbn1*$^{C1039G/+}$ mice. Histologic analysis of lung in wild-type mice (**A**) shows normal airspace caliber. *Fbn1*$^{C1039G/+}$ mice treated with placebo (**B**) show diffuse distal airspace widening (example delineated with arrows). Distal airspace caliber is reduced in *Fbn1*$^{C1039G/+}$ mice treated with losartan (**C**). Scale bars, 500 μm. (**D**) Average mean linear intercept, a marker of airspace caliber, is greater for placebo-treated *Fbn1*$^{C1039G/+}$ mice than for untreated wild-type and losartan-treated *Fbn1*$^{C1039G/+}$ mice. $*P < 0.01$, $**P < 0.001$.

of strokes in both adults and children. Given its exceptional tolerance profile in all age groups, we conclude that a prospective clinical trial in patients with MFS is indicated. Furthermore, this study is illustrative of the promise that enhanced identification of disease genes in the post–genome sequencing era will have a pronounced impact on medicine. Disease gene discovery is but an obligate first step in the process of making animal models, interrogating pathogenesis, and deriving unanticipated disease mechanisms and rational treatment strategies.

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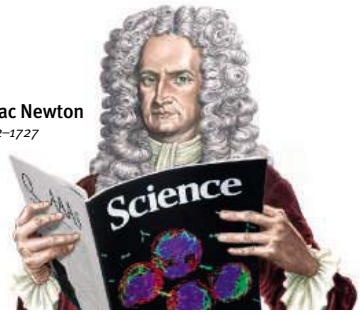

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Division of Cancer Biology

The Department of Health and Human Services(DHHS), National Institutes of Health(NIH), National Cancer Institute (NCI), is seeking a Deputy Director, Division of Cancer Biology (DCB), to participate in the scientific and administrative management of the Division. DCB has principal responsibility for the planning, direction, coordination and evaluation of a broad program of extramural research in cancer biology and etiology, with six Branches focused on cancer cell biology, tumor biology and metastasis, cancer immunology and hematology, DNA and chromosome aberrations, cancer etiology, and structural biology and biotechnology with an annual budget of over $800 million that supports grants, cooperative agreements, contracts and operating expenses. The Deputy Director participates in the planning of a coordinated program in the areas of cancer etiology, biology and immunology and is directly responsible for conducting a continuous review and evaluation of the Division's operations as they pertain to a broad program of grant supported activities designed to increase knowledge of cancer biology. The Deputy Director, DCB, will be appointed at a salary commensurate with his/her qualifications and experience. Full federal benefits include, leave, health and life insurance, retirement savings plan (401K equivalent).

Qualifications Required: The applicant must have a doctoral level degree (Ph.D.) Or medical degree (M.D.) with significant research experience. The applicant currently must be an experienced Senior Scientist and/or Science Administrator with considerable expertise and demonstrated recognition for achievements related to cancer biology research and documented experience in program planning, implementation and oversight and in grants management. The applicant must have significant administrative, managerial and supervisory experience.

How to apply: Applicants should send a brief biography, curriculum vitae, bibliography and the names and addresses of four references to: **Dinah A. Singer, Ph.D., Director, Division of Cancer Biology, National Cancer Institute, 6130 Executive Blvd., Building EPN, Room 5044, Rockville, MD 20852-7380.**

If you need additional information, please call **Mr. Stephen White at (301) 594-8905 or Mrs. Bridgette Tobiassen at (301) 594-8786. DEADINE FOR RECEIPT OF APPLICATIONS: May 31, 2006.**

The University of Texas M. D. Anderson Cancer Center

Department of Clinical Cancer Prevention

The M. D. Anderson Cancer Center has been ranked as one of the top two cancer centers in the U.S., for the last 10 years. The M. D. Anderson Cancer Center is known for excellence in collaborative translational, clinical and prevention research and cross-disciplinary training to support pre- and post-doctoral studies.

The Division of Cancer Prevention and Population Sciences, which consists of four Departments (Behavioral Science, Clinical Cancer Prevention, Epidemiology, and Health Disparities Research) is renowned for its innovation and leadership. Clinical Cancer Prevention is a major area of emphasis for the Mission of the University of Texas M. D. Anderson Cancer Center to "Make Cancer History™". We currently seek:

Chair Person

Clinical Cancer Prevention

The Chair of Clinical Cancer Prevention will be responsible for developing and supporting a highly innovative and collaborative Clinical Cancer Prevention Program encompassing Clinical Genetics, Early Detection, Interventional Cancer Prevention including nutritional, and chemoprevention approaches, and Survivorship. This will include maintaining and extending existing efforts as well as developing and implementing new programs. The successful candidate will exhibit the leadership, vision, and breadth of knowledge required to fulfill these goals. In particular, demonstrated expertise in consensus building and administration of large-scale interdisciplinary collaborative efforts is required. Experience in implementing and coordinating nutritional, chemo or vaccine prevention trials would be an asset. The successful candidate will be responsible for the mentorship and career development of currently existing faculty as well as for new recruits. A unique opportunity exists to develop educational programs for oncology trainees in Cancer Prevention. The successful candidate should have an M.D. and/or Ph.D. with a nationally and internationally recognized interactive and collaborative research program in Clinical Genetics, Early Detection, Interventional Cancer Prevention, or Survivorship. The Department of Clinical Cancer Prevention has recently moved into state-of-the-art clinical, laboratory and office space. With the appropriate leadership, the Cancer Prevention Center is poised to become a pivotal institutional resource for the conduct of translational prevention studies. Generous resources are available for expansion of the Departmental role and for the research programs of the successful candidate.

M. D. Anderson Cancer Center offers a highly attractive recruitment package, active graduate and postdoctoral training programs, and the unrivalled scientific environment of the Texas Medical Center. Please forward letter of intent including discipline of interest, research goals, CV, and complete contact information for three references to:

Alice Burnett
Office of EVP and Chief Academic Officer - Box 0113
The University of Texas M. D. Anderson Cancer Center
1515 Holcombe Boulevard
Houston, TX 77030

Closing date for applications: May 31, 2006

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

M. D. Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas M. D. Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

U.S. GENOMICS

U.S. Genomics is seeking highly motivated scientists to work in a multi-disciplinary team on an advanced product development program for the U.S. Dept. of Homeland Security Advanced Research Projects Agency (HSARPA). USG's proprietary technology for stretching and identification of DNA fragments without amplification offers a unique advantage for threat detection with high sensitivity and specificity. U.S. Genomics has been awarded a Phase II contract by HSARPA to continue development of the Company's sophisticated biological sensor for biodefense applications. Under the 18-month, $16.2 million contract, funded by HSARPA's Bioagent Autonomous Networked Detectors (BAND) program, U.S. Genomics will complete technology and prototype development of its system for the detection and identification of airborne pathogens using its DNA mapping technology.

- **2006S-04RG – Ph.D. Staff Scientist, Environmental Testing:** Chemical Engineering or Biophysics.
- **2006S-06RG – Ph.D. Staff Scientist, Human Diagnostics:** Microbiology or Molecular Biology.
- **2006S-05RG – Ph.D. Staff Scientist:** Microbiology.
- **2006S-18JL – Ph.D. Engineer or Applied (Bio)Physicist:** Mechanical/biomedical engineering.
- **2006S-17EM - Ph.D. Staff Scientist:** Molecular Biophysics, Physical Chemistry or Polymer Physics.
- **2006S-20EM - Ph.D. Staff Scientist:** Biochemist or Chemical Engineer with specialization in Protein Chemistry, Nucleic Acid Chemistry or Bioconjugation.

To learn more about these positions and apply, visit our Website at **www.usgenomics.com/index.php**. In the upper right hand corner select "Careers" and search for the job code listed.

U.S. Genomics offers a comprehensive salary and benefits package. All positions are located in Woburn, MA. Relocation assistance is available.

We are an Equal Opportunity Employer.

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

Universität Stuttgart

Fraunhofer Gesellschaft

An der Universität Stuttgart ist in der Fakultät für Maschinenbau die

Professur für Grenzflächenverfahrenstechnik (W3 mit Leitungsfunktion)

(Nachfolge Prof. Dr. techn. Herwig Brunner) zum nächst möglichen Termin zu besetzen. Gleichzeitig sucht die Fraunhofer-Gesellschaft in Personalunion die

Institutsleiter/Institutsleiterin am Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik in Stuttgart

Die Universität Stuttgart und das Fraunhofer-Institut kooperieren auf den genannten Fachgebieten in Lehre und Forschung. Mit der Verknüpfung von Universitätsprofessur und Institutsleitung sowie der gemeinsamen Nutzung von Ressourcen sollen die praxisnahe Ausbildung von Studierenden und Graduierten sowie die wirtschaftswirksame Umsetzung von Forschungsergebnissen gefördert werden.

An der Universität Stuttgart leitet die gesuchte Persönlichkeit das Institut für Grenzflächenverfahrenstechnik IGVT und vertritt das Gebiet der Grenzflächen- und Oberflächentechnik. Dieses umfasst die Grundlagen der Struktur und des Aufbaus von Grenzflächen, deren Charakterisierung, Modifizierung und Funktionalisierung sowie die Erarbeitung von Anwendungen, insbesondere in den Gebieten der Biotechnologie und Medizintechnik bis hin zu pharmazeutischen Bereichen.

In der Lehre vertritt die gesuchte Persönlichkeit das Gebiet der Grenzflächen- und Oberflächentechnik mit Schwerpunkt Medizinische Verfahrenstechnik und Bioverfahrenstechnik, Nano-(Bio-)Technologie. Das Lehrgebiet Grenzflächenverfahrenstechnik ist in den Studiengang Verfahrenstechnik und Technische Kybernetik mit den Vertiefungsfächern Bioverfahrenstechnik, Biomedizinische Verfahrenstechnik sowie in die Ausbildung der Technische Biologie eingebunden.

Die Forschungsaktivitäten sollen sich auf die Bereiche der Oberflächenanalytik, der Oberflächencharakterisierung und -funktionalisierung sowie der Biofunktionalisierung erstrecken, insbesondere für diagnostische und medizinische bis therapeutisch einsetzbare Systeme (medical devices).

Das Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB arbeitet auf den Gebieten Funktionalisierung und Charakterisierung von Grenzflächen und Materialoberflächen für Technik und Medizin, 3-D Zellsysteme sowie der molekularen Biotechnologie für Diagnostik, Pharma, Feinchemie und zusätzlich der nachhaltigen Bioverfahrenstechnik für Industrie, urbane Infrastruktur und Umwelt. Dabei werden überwiegend industriellen Kunden Forschungsdienstleistungen von der Idee über die Produkt-, Material- und Verfahrensentwicklung bis hin zur Prototypenherstellung angeboten. Das IGB beschäftigt derzeit ca. 150 Mitarbeiter bei einem Gesamthaushalt von rund 9 Mio. Euro, wovon etwa 6 Mio. Euro aus Projekten extern finanziert werden. Die Leitung des Fraunhofer-Instituts beinhaltet die Gesamtverantwortung für die strategische Entwicklung der Geschäftsfelder, die Sicherstellung hoher wissenschaftlicher Qualität der FuE-Arbeiten, die Akquisition der Forschungs- und Entwicklungsprojekte und die Betriebsführung des Instituts (www.igb.fraunhofer.de).

Gesucht wird eine Persönlichkeit, die auf den genannten Gebieten hervorragend ausgewiesen ist und das Fachgebiet in der Wissenschaft ebenso kompetent vertreten kann wie gegenüber Forschungsförderern und industriellen Vertragsforschungspartnern.

Die Universität Stuttgart und die Fraunhofer-Gesellschaft möchten den Anteil der Frauen im wissenschaftlichen Bereich erhöhen und sind deshalb an Bewerbungen von Frauen besonders interessiert. Schwerbehinderte werden bei gleicher Eignung vorrangig eingestellt.

Es gelten die Einstellungsvoraussetzungen des §47 Landeshochschulgesetz. Gemäß §50 Absatz 1 Landeshochschulgesetz ist das Dienstverhältnis bei einer ersten Berufung in ein Professorenamt normalerweise auf höchstens vier Jahre befristet; Ausnahmen sind möglich. Bewerbungen mit den üblichen Unterlagen sind bis zum 15. Mai 2006 zu richten an den
Prorektor für Struktur der Universität Stuttgart
Herrn Prof. Dr.-Ing. Wolfgang Ehlers
Institut für Mechanik (Bauwesen)
Universität Stuttgart, 70550 Stuttgart
und an den
Präsidenten der Fraunhofer-Gesellschaft
Herrn Prof. Dr.-Ing. Dr. h. c. mult. Hans-Jörg Bullinger
Postfach 20 07 33, 80007 München

University of Bergen

is a city university. Parts of the campus are in fact situated in the town centre. We have about 17.000 students and nearly 3000 employees. UiB is renowned for its research which holds a high European standard and we have three Centres of Excellence (CoE). The University of Bergen has a strong international profile which entails close co-operation with universities all over the world.

Faculty of Matematics and Natural Sciences - Departement of Biology

Professor/Associate Professor in Developmental Biology of Fish (Early Life Stages)

The professor shall perform basic research on fundamental biological questions related to developmental processes during early stages of fish development with emphasis on environmental, genetic and physiological adaptation and regulation of growth and development through embryo, larval and juvenile stages. Start-up funding will be made available to help the successful candidate quickly becoming established in the position. More at www.uib.no/stilling and www.bio.uib.no. Submit application in 5 copies, sorted in 5 bundles, to **Department of Biology, University of Bergen, PO Box 7800, NO-5020 Bergen, Norway, by 20 April 2006.**

For more information - see the web site: uib.no/stilling

CICERO ab

Faculty Position
NEUROSCIENTIST

The Department of Anatomy and Cell Biology at Downstate Medical Center invites applications for a tenure-track ASSISTANT PROFESSOR position. The successful candidate is expected to develop an independent, extramurally-funded research program in the neurosciences and to participate in teaching medical students as well as training graduate students. Preference will be given to candidates with current extramural funding and prior teaching experience or training in neuroanatomy.

Curriculum vitae, a brief description of previous and anticipated research, and the names of three references should be sent to:

Dr. M.A.Q. Siddiqui
Professor and Chair
Department of
Anatomy and Cell Biology
State University of New York
Downstate Medical Center
450 Clarkson Avenue, Box 5
Brooklyn, NY 11203

Fax: 718-270-3732
E-mail: MAQ.Siddiqui@Downstate.edu

SUNY DOWNSTATE Medical Center

SUNY Downstate is an EOE

Technical University of Denmark

Professor and Director

DTU announces a position as full professor in Electron Microscopy. The professor will lead research in Electron Microscopy and at the same time serve as Director for our new Center for Electron Nanoscopy.

We are in the process of establishing an advanced center for electron microscopy, which will complement our strong activities within nanotechnology and materials science. The center, which is made possible by a generous donation by the A.P. Moller Foundation, will be equipped with a total of six microscopes of which two will be absolute state of the art TEM's with sub-Ångstrom resolution, energy loss spectroscopy, and 3D structural analysis facilities. Moreover, one of the TEM's will be modified into an environmental TEM. CEN is expected to be fully operational in the fall of 2007.

We seek a candidate with the highest academic qualifications and demonstrated abilities in leading advanced research at an international level related to electron microscopy.

For more information contact Dean of Research, Prof. Kristian Stubkjaer (forskningsdekan@adm.dtu.dk, direct telephone +45 4525 1008).

The full text of the announcement can be seen on DTU's homepage at www.dtu.dk/vacancy

Application deadline: 2nd May 2006 at 12.00.

Scripps Institution of Oceanography
Faculty Position in Marine Organismal Environmental Physiology

The Scripps Institution of Oceanography of the University of California at San Diego invites applications at the Assistant, Associate, or Full Professor level (tenure track or tenured) in the area of environmental physiology of marine animals with emphasis on whole organism or organ-system function. Applicants must hold a Ph.D. degree or equivalent and will be expected to teach, supervise graduate research, conduct an active research program, and participate in administrative functions at SIO and UCSD. Assistant-level applicants will be expected to show evidence of their potential by a publication record appropriate for their experience. More senior applicants must show evidence of a strong research record in their specialty. The level will depend on the experience of the successful applicant. Salary will be based on the University of California pay scale.

The closing date for applications is **May 15, 2006**, with interviews to begin immediately after the search is closed. Applicants should send a letter including descriptions of their teaching experience, research interests, a list of publications, and the names of at least five potential referees to: **Chair, SIO Graduate Department, 0208, Scripps Institution of Oceanography, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0208, USA.**

UCSD is an Equal Opportunity Employer with a strong institutional commitment to excellence through diversity.

FACULTY POSITION ANNOUNCEMENT
BIOMOLECULAR IMAGING

THE DEPARTMENT OF MEDICAL PATHOLOGY AND LABORATORY MEDICINE
UNIVERSITY OF CALIFORNIA, DAVIS

The Department of Medical Pathology and Laboratory Medicine at the University of California, Davis, announces a search for a full time faculty position (Associate/Professor rank - level and salary commensurate with qualifications and experience). Responsibilities include providing prominent leadership in the establishment and development of the biomolecular imaging and characterization program at the University of California, Davis, Medical Center. This recruitment will be a part of the Center for Biophotonics Science and Technology Center at UC Davis and will be closely integrated with the Stem Cell Research Program at UC Davis. Requires funded independent research programs and familiarity in areas related to the use of single molecule microscopy, optical probe development and characterization using Raman spectroscopy or other optical modalities for imaging and characterization of cellular and subcellular structure and function. Emphasis either on basic biophysical questions or on translational applications is most appropriate. A Ph.D. is required. Candidates working in any area of biophotonics research are encouraged to apply. Special consideration will be given to applicants working in the areas of biomedical optics and cell surface characterization, with particular emphasis on the application of these technologies in stem cell research. The successful candidate will be expected to perform research/creative work, teaching of medical students, residents, fellows, and graduate students, university and public service. The applicant should have a strong background in teaching of medical students, residents, fellows and graduate students and research that would qualify them for a position at the Associate/Full Professor level at the University of California, Davis. This position includes office and research space, and access to a large number of core facilities at the University and the UC Davis Medical Center.

Applicants should submit (1) a letter of interest describing research and teaching background; (2) a curriculum vitae, including reprints of three selected recent publications; and (3) five references (including name, address and phone number). For full consideration, applications must be received by **May 1, 2006**. Please forward curriculum vitae and the names of five references to: **Anthony Cheung, Ph.D, Professor and Vice Chair, Research, Department of Medical Pathology and Laboratory Medicine, University of California, Davis Medical Center, 4400 V Street, Sacramento, CA 95817.**

The University of California, Davis, is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to the achievement of diversity.

University of Missouri Health Care

Research Faculty Position

The University of Missouri School of Medicine Department of Surgery is seeking outstanding candidates for two research faculty positions. Rank and appointment status are contingent upon qualifications.

The candidate should have experience in a field of research in which surgeons interface. This includes, but is not limited to, cancer, heart disease, inflammation, wound healing, trauma, and vascular biology. Candidate must demonstrate evidence of current or future potential for federal funding, show interests in teaching medical students and surgical residents, and have the desire to interact with surgical researchers in developing a vigorous, extramurally funded research program.

Applicants should send curriculum vitae to: **Steve Eubanks, M.D., Chairman, Department of Surgery, University of Missouri-Columbia, Health Sciences Center, One Hospital Drive, Columbia, Missouri 65212.**

E-mail: grotewielln@health.missouri.edu

Equal Opportunity/ Affirmative Action/ ADA Employer

Visit the Department of Surgery's Web site at http://www.surgery.missouri.edu/. Go to "Practice Opportunities".

National Oceanic and Atmospheric Administration (NOAA)

Director, Chemical Sciences Division (CSD)

(A Senior Executive Service Position in the Federal Government)
Vacancy Announcement NOAA#06-11

Department of Commerce (DOC)
National Oceanic and Atmospheric Administration (NOAA)
Office of Oceanic and Atmospheric Research (OAR)
Boulder, Colorado
$109,808 - $152,000 annually

The candidate selected for this position plans, leads, and manages the scientific research of the ESRL Chemical Sciences Division. The scientific goal of CSD is to improve the understanding of the chemical and related meteor logical processes in Earth's Atmosphere that affect climate, air quality, and the stratospheric ozone layer, and to assess and communicate the current state of scientific understanding on those topics to NOAA's information customers in government, industry and the public. ***The applicant must possess the following:***

1. Broad background and direct experience in research and development in a combination of disciplines (e.g., atmospheric chemistry, physics, meteorology, and atmospheric dynamics) to be able to conceive, design, direct, and evaluate theoretical and experimental atmospheric research studies of a complex and large-scale nature, with an ability to provide leadership regarding phenomena and issues that encompass multiple disciplines and multiple environmental issues.

2. A history of research experience that places the incumbent as a recognized world scientific or technical leader in one or more of the above research and development fields.

3. High level competence in the chemical or physical sciences sufficient to perceive complex problems in broad perspective, to infuse ideas, and to participate meaningfully in discussions and evaluation of program areas in atmospheric research.

4. Demonstrated ability to perform personal senior-level research with particular emphasis on the atmosphere, and to publish significant research findings in refereed literature.

Please contact **Norma Hughes at 301/713-6307** for an announcement package **(Internet: address: norma.j.hughes@noaa.gov),** including mailing instructions—referring to the announcement number -OR- you may access the entire full-text vacancy from NOAA's Executive Resources Homepage (see below).
Incomplete applications will be returned.
http://www.hr.noaa.gov/er-home.htm
This vacancy will close on May 25, 2006
"NOAA Values a Diverse Workforce and is an Equal Opportunity Employer"

HOSPITAL FOR SPECIAL SURGERY

CORNELL UNIVERSITY
Joan and Sanford I. Weill Medical College

Faculty Position
Tissue Engineering, Regeneration and Repair Program

The Hospital for Special Surgery, in conjunction with the Weill Medical College of Cornell University, is undertaking a major initiative in tissue engineering and regenerative medicine. The Tissue Engineering, Regeneration and Repair (TERR) Program at Hospital for Special Surgery is seeking an individual for a tenure-track research position at the Associate or Full Professor level, with a joint academic appointment at Cornell University. An endowed chair has been established to help support this position. The candidate will be responsible for developing an innovative and competitive program of research in tissue engineering and remodeling of soft tissues of the musculoskeletal system. Preference will be given to candidates having a strong extramurally funded research program. Candidates should have expertise in one or more of the following areas: developmental biology, mesenchymal stem cell biology, or cell-signal transduction by engineered extracellular matrices. Hospital for Special Surgery is a leading hospital for orthopaedics and rheumatology and for basic, applied and translational musculoskeletal research, including treatment of arthritis and rheumatic diseases, bone and cartilage physiology, skin and wound healing, osteoporosis and sport injuries. The TERR Program (**http://hss.edu/Research/Programs/**) interfaces with existing research Programs in Musculoskeletal Integrity, Autoimmunity and Inflammation, and Arthritis and Tissue Degeneration, and with investigators at the adjacent Memorial Sloan Kettering Cancer Center, Rockefeller University, City University of New York, and Cornell University in Ithaca, New York.

Applicants should send a CV, a brief summary of research accomplishments and future objectives, and the names and addresses of three references to:

Dr. Peter A. Torzilli
Hospital for Special Surgery
535 East 70th Street
New York, NY 10021

An Equal Opportunity/Affirmative Action Employer.

WAKE FOREST UNIVERSITY
SCHOOL of MEDICINE

FACULTY POSITIONS
For Basic Scientists in Wound Healing, Repairs and Regeneration
Wake Forest University Medical Center

The Department of Plastic and Reconstructive Surgery at the Wake Forest University School of Medicine has significantly expanded its research space and is undertaking a revolutionary new initiative in wound healing and tissue repair/regeneration. The Department is hiring two additional research faculty members (Ph.D., M.D.). These positions will work in direct collaboration with clinical surgeons and tissue engineers.

The ideal candidates must possess experience and qualifications in their area of expertise, be highly motivated, be able to work and communicate effectively with others, and be interested in moving ideas from conception to clinical implementation. Each scientist will be a critical component of world class multidisciplinary teams that will work together to define fundamental principles, apply these directly to clinical problems, and pursue translational research. We are seeking basic scientists in the following areas.

ANGIOGENESIS: A highly skilled individual with solid background in angiogenesis, particularly as it relates to wound healing, pathologic growth and congenital malformations, aging, and/or neoplasm. A significant understanding of clinical and laboratory protocol development is desirable.

CRANIOFACIAL BIOLOGY: A highly skilled individual is sought with a solid background in craniofacial biology, developmental biology of the cranium, and/or pathologic growth and congenital malformations. A significant understanding of clinical and laboratory protocol development is desirable.

The Department of Plastic and Reconstructive Surgery at Wake Forest University Medical Center has an international reputation of highly innovative productivity and translational research. Individuals selected for these two positions will receive a competitive salary, significant job security, and the ability to be involved with and work in a revolutionary environment. Team members may derive financial gain from any commercial development.

Applicants should E-mail or mail a detailed statement of research interest and copy of their CV and three references to: **Dr. Louis Argenta, Professor, Department of Plastic and Reconstructive Surgery, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157-1075; largenta@wfubmc.edu**.

Wake Forest University is an Equal Opportunity Employer and actively seeks applications from women and minorities.

Editor - Nature China

Nature Publishing Group, the publisher of *Nature*, is pleased to announce the launch of *Nature China* in October 2006.

Nature China will be an electronic website highlighting the best research from mainland China and Hong Kong. The aim is to publish short articles highlighting the scientific significance of research published in the scientific literature. As part of this exciting new publishing venture, we are now seeking an Editor to develop *Nature China* from the initial design stage through to launch and future developments. The post, initially running until October 2007, will be based (after a period of training in our London office) in our Hong Kong office.

The Editor will have a Ph.D. in physics, chemistry or a life science, with demonstrable research achievements. Though postdoctoral experience is preferred (not required) emphasis will be placed on broadly trained applicants. Key elements of the position include the selection of manuscripts and writing concise summaries highlighting the scientific significance of the chosen article. Candidates must demonstrate a good understanding of research communities in China as well as being fluent in English and preferably Mandarin.

This is a demanding and extremely stimulating role, which calls for a keen interest in the practice and communication of science. The successful candidate will therefore be dynamic, motivated and outgoing, and must possess excellent interpersonal skills. The salary and benefits will be competitive, reflecting the critical importance and responsibilities of this position.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), **Three** Research Highlight style pieces (350 words or less) on three recent papers (from mainland China or Hong Kong) from related literature, and a brief cover letter explaining why they have chosen these manuscripts, their salary expectations and their interest/suitability for the post.

Please send applications, clearly stating reference number NPG/LON/393 to: Rebecca Innes, Personnel Assistant at londonrecruitment@macmillan.co.uk

Closing date: 30th April 2006

nature publishing group npg

THE INSTITUTE FOR ADVANCED LEARNING AND RESEARCH

Director of Research and Development at the Institute for Advanced Learning & Research in Danville, Virginia

Virginia Tech seeks a Director of Research and Development for the Institute for Advanced Learning & Research (IALR) to oversee Research and Development programs. IALR (*www.ialr.org*) is a mission-oriented establishment focusing on the economic diversification and revival of Southside Virginia. This purpose is being accomplished through research, innovation, technology development/transfer, education and outreach, in a multidisciplinary setting, in partnership with: Virginia Polytechnic Institute & State University (Virginia Tech), Averett University, Danville Community College, Future of the Piedmont Foundation, Pittsylvania County, City of Danville, local K-12 school systems, government agencies and local businesses. The IALR facilities include modern engineering and plant biotechnology laboratories, classrooms and state-of–the-art conferencing facilities. IALR faculty are part of a vibrant Virginia Tech (*www.vt.edu*) engineering and life sciences community encompassing interdisciplinary programs in mechanical engineering, robotics, polymer sciences, and plant genomics. Currently four specifics areas of research are ongoing. They are: 1. The Advanced and Applied Polymer Processing Institute (APPI); 2. The Joint Unmanned Systems Testing, Experimentation, and Research (JOUSTER); 3. The Virginia Institute for Performance Engineering and Research (VIPER); and 4. The Institute for Sustainable and Renewable Resources (ISSR). These programs have garnered over $15 M in external research funding over the past two years. The successful candidate as Director of Research and Development will be an active member of the senior leadership team of IALR and may be considered for a tenure-track position in an appropriate academic department at Virginia Tech.

Application procedure: Interested candidates must apply on-line to posting number 060026 at www.jobs.vt.edu. Applicants should include: 1. A cover letter summarizing their intent and qualifications, 2. A current CV, and 3. Names of at least three references including names, affiliations and contact information (email address and telephone number). Inquires should be directed to Dr. James Blair, Chair of the Search Committee: Office of the Vice President for Research, 301 Burruss Hall, Virginia Tech, Blacksburg, VA 24061. Phone: 540/231-5410; Email: *jblair@vt.edu*.

Virginia Tech has a strong commitment to the principle of diversity and, in that spirit, seeks a broad spectrum of candidates including women, minorities, and people with disabilities. Individuals with disabilities desiring accommodations in the application process should notify Debbie Nester at Virginia Tech, phone 540/231-5410.

VirginiaTech
Invent the Future

DIRECTOR
Chemical Sciences, Geosciences and Biosciences Division

The US. Department of Energy, Office of Science, Office of Basic Energy Sciences is seeking candidates for the position of the Director, Chemical Sciences, Geosciences and Biosciences Division. This position is a Senior Executive Service position located in Germantown, Maryland. The incumbent of this position provides leadership and direction in establishing vision, strategic plans, goals, and objectives for the research activities in the area of responsibility; plans, develops, and implements vital, productive, forefront research programs conducted in DOE laboratories, universities, and other public and private institutions; assures adequate financing for the activities, sets priorities for activities, and apportions available funding among them; manages the budget in accordance with prescribed practices set forth by the Office of Science and the DOE; reviews and makes final approvals on staff recommendations concerning research agreements and individual proposals for research projects; implements rigorous merit evaluation for all new and ongoing activities in accordance with the federal requirements for the grant program and published guidelines for DOE laboratory programs and facilities; assists in the formulation and management of new programs and policies; coordinates research activities with those of other Federal agencies; represents the Division, the Office, and DOE on professional societies, committees, task forces, etc.; supervises a staff of professional, scientific and administrative employees.

For more detailed information on this position, go to the website: **http://jobsearch.usajobs.opm.gov/**. Go to Search Jobs and in the Key Word Search box type **06PH-ES-22-001**.

For information on how to apply for this position, please follow the instructions as stated in the following website: **http://jobsonline.doe.gov**. **NOTE: Applications must be submitted online**.

IFOM
FIRC INSTITUTE OF MOLECULAR ONCOLOGY

IFOM-IEO CAMPUS
For Molecular Oncology
MILAN, ITALY
www.ifom-ieo-campus.it

EUROPEAN INSTITUTE OF ONCOLOGY

Two of the main Cancer research Institutions in Italy, the FIRC Institute of Molecular Oncology (IFOM) and the European Institute of Oncology (IEO) have expanded and integrated their research activities into a common campus. The IFOM-IEO Campus is also home to the PhD program of the European School of Molecular Medicine (www.semm.it). Central services include Animal Facilities (mouse, zebrafish and C. elegans), Imaging, Protein and Antibody Production, DNA and Tissue Microarrays, DNA Sequencing, Real-time PCR, Bioinformatics. Open structure laboratories foster communication between groups. We are now calling for applications for the position of:

HEAD OF PROTEOMICS

The successful candidate will manage both his/her own research group and the Proteomics Facility in the Campus. Commensurate packages will be provided for each activity, including equipment and personnel. As a Group Leader in the Campus, the candidate will be expected to develop an internationally competitive line of research in any area pertinent to cancer biology, diagnostics or therapy. As a Facility Director, the candidate will guarantee access to proteomics-based solutions to other research groups in the Campus. The Facility is expected to maintain a state-of-the-art technological infrastructure over time. The Campus provides a vast potential for scientific interactions. Candidates should have extensive experience in proteomics and a proven track-record in running a competitive research group.

DEADLINE FOR APPLICATIONS: June 30, 2006.

Applications should be sent by e-mail only to **search-proteomics@ifom-ieo-campus.it** and should include: CV, publication list, statement of Achievements and Interests (max. 2 pages), names and e-mail addresses of 4-5 referees. Applicants should also ask their referees to directly send their letters to the same e-mail address.

The IFOM-IEO campus is an equal opportunity employer. We encourage applications from women and will implement measures required to place all scientists in a situation of equal competitiveness.

Max Planck Institute for Neurological Research

MAX-PLANCK-GESELLSCHAFT

with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne, Director Prof. Dr. D. Yves von Cramon

The Max Planck Institute for Neurological Research invites applications for four Group Leader positions

Selbständige Nachwuchsgruppe der Max-Planck-Gesellschaft (Independent Junior Research Group)

The Max Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Medical Faculty of the University of Cologne has opening for four Group Leader positions, funding for a research group (Selbständige Nachwuchsgruppe der Max-Planck-Gesellschaft / Independent Junior Research Group) dedicated to neurological and oncological research. While there is no particular restriction on the research area, there is the expectation that the successful candidate will interact synergistically with other groups at the Institute.

Currently, the following technologies / methods are available at the Institute: **human brain PET** (EXACT-HR, HRRT), **animal PET** (μPET) **and animal MRI** (4.7T, 7.0T, 11.7T), **optical imaging** (bioluminescence, fluorescence and laser scanning microscopy) **autoradiography, electron microscopy, histology, immunohistochemistry and invasive imaging / neuromonitoring** (microdialysis, subdural EEG). In 2006, a **combined human brain PET-MR-System** will be installed.

Cologne and its rich scientific environment in North-Rhine-Westphalia provide many opportunities of interactions with the University of Cologne and the Universities in the region, including the teaching of courses.

The Group Leader positions and funding are guaranteed for five years with the possibility of extension.

The Max Planck Society and the University of Cologne aim to increase the representation of women and therefore explicitly encourage applications from female scientists.

Deadline for applications is May 12, 2006. Further information can be obtained through

Max Planck Institute for Neurological Research
Prof. Dr. Rudolf Graf, Deputy Managing Director
Gleueler Str. 50, 50931 Köln
Tel.: +49 (0)221 4726-201
Fax: +49 (0)221 4726-203
Email: rudolf.graf@nf.mpg.de

University Of Nebraska Lincoln

Tenure Track Assistant Professor for Instructional Biochemistry

The Center for Biological Chemistry and Department of Biochemistry at the University of Nebraska-Lincoln seek a highly motivated, well trained individual to fill a 12-month, 80% teaching/20% research, tenure track position for instruction in Biochemistry. The primary responsibility for the individual chosen will be instruction in a rapidly growing undergraduate Biochemistry program. Active involvement in student recruitment and retention, undergraduate student assessment, and career counseling are also expected. A proven record of teaching excellence at the college level, strong organizational and communication skills, and a strong background in Biochemistry research, including post-doctoral training (or its equivalent), are required along with a long-term commitment to providing high quality undergraduate instruction. The individual will be expected to apply for internal and external funding to support educational programs. A PhD in Biochemistry or a closely related field is required. The Department offers B.S., M.S. and Ph.D. degrees in Biochemistry and is home to internationally recognized research programs in diverse areas of biochemistry and molecular biology (**http://biochem.unl.edu/**). Review of applications will begin **April 27, 2006**, and continue until the position is filled or the search is closed.

Applicants should go to **http://employment.unl.edu/** (requisition **#060194**) and complete the Faculty/Administrative Information form and then send a complete application file, consisting of a statement of research interests, CV and arrange for three letters of recommendation be sent to: **Search Committee Chair, Assistant Professor for Instructional Biochemistry, N200 Beadle Center, University of Nebraska-Lincoln 68588-0664 (USA)**.

UNL is committed to a pluralistic campus community through affirmative action and equal opportunity and is responsive to the needs of dual career couples. We assure reasonable accommodation under the Americans with Disabilities Act. Contact ***Holly Henrichs*** *at* ***(402) 472-4742*** *or* ***hhenrichs1@unl.edu*** *for assistance.*

CENTER FOR STATISTICAL GENETICS

University of Michigan

Departments of Ophthalmology and Visual Sciences, Human Genetics, and Biostatistics

The Departments of Ophthalmology & Visual Sciences, Human Genetics, and Biostatistics at the University of Michigan jointly invite applications for a tenure-track faculty position in statistical genetics. Both junior and senior candidates are encouraged to apply. Tenured appointments are available to outstanding candidates. Areas of interest include genetic epidemiology, bioinformatics, population genetics and complex diseases. Generous salary and start up support are available. Applicants should have a doctoral degree, demonstrate outstanding research productivity or promise in statistical genetics, and have an interest in applications to the genetics of vision-related traits.

Inquiries and applications to: Anand Swaroop PhD (734-615-2246, swaroop@umich.edu) and Gonçalo Abecasis (734-763-4901, goncalo@umich.edu), Co-Chairs, Statistical Genetics Search Committee, University of Michigan Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105. Information about the Departments is available at www.med.umich.edu/kec/, www.med.umich.edu/hg/, and www.sph.umich.edu/biostat/. Applicants should send CV and statement of research interests. Applicant review will begin April 17th and continue until the position is filled.

The University of Michigan is an affirmative action/equal opportunity employer.

Postdoctoral Fellowships for Research on Adult Stem Cells

Tulane Center for Gene Therapy

The Tulane Center for Gene Therapy is again offering a program of postdoctoral fellowships for research on adult stem/progenitor cells for non-hematopoietic tissues. The aim of the program is to prepare postdoctoral fellows for careers as independent academic scientists. Applicants can select from research projects that range from basic biology of stem cells to their application to animal models for genetic diseases of children, cancers, heart disease, lung disease, stroke, Alzheimer's disease, spinal cord injury, parkinsonism, and diabetes. The Center is a leader in isolation and characterization of stem/progenitor cells from bone marrow referred to as MSC, and conducts an NIH sponsored program to distribute fully characterized samples to other investigators. The Center includes a GMP level facility for preparing stem/progenitor cells for clinical trials. The Center is housed in a modern research building, it has a staff of over 70, and it is supported by ten core laboratories. Two members of the faculty also conduct research with stem/progenitor cells in non-human primates at the Tulane University Regional Primate Center. Qualified applicants should have a Ph.D. degree or equivalent from a well-recognized college or university.

For details, visit web site: **www.som.tulane.edu/gene_therapy/**. Please send (1) curriculum vitae, (2) brief summary of research interests, and (3) two or more letters of recommendation to:

Dr. Darwin J. Prockop, Director
Tulane Center for Gene Therapy
Tulane University Health Sciences Center
1430 Tulane Avenue, SL-99
New Orleans, LA 70112

Fax: 504-988-7710
E-mail: dprocko@tulane.edu

Tulane is an Equal Opportunity Employer.

Baxter

Director, Solutions Research

Baxter Healthcare Corporation assists healthcare professionals and their patients with the treatment of complex medical conditions, including cancer, hemophilia, immune disorders, kidney disease and trauma. The company applies its expertise in medical devices, pharmaceuticals and biotechnology to make a meaningful difference in patients' lives.

In this new position, the Director, Solutions Research will be responsible for the identification and evaluation of new peritoneal dialysis solution technologies, and management of a Peritoneal Dialysis solutions research group consisting of Ph.D. and Master level scientists. The candidate will be expected to lead projects from inception through proof of principle, be able to articulate the clinical and business value of such projects to both internal management groups and external customers, as appropriate.

The ideal candidate is required to have a Ph.D. in Physiology, Biochemistry, Pharmacology or equivalent. Post-doctoral experience desirable. This individual will have a demonstrated ability to effectively direct a multi-faceted research program. Scientific expertise/training that would permit rapid appreciation of transport kinetics and metabolism of osmotic agents is necessary. Knowledge of the drug development process, especially in the discovery and pre-clinical phases is essential. Demonstrated independent decision making and problem solving abilities is critical. Excellent written, verbal and interpersonal communication skills is required. The ability to formulate creative and innovative concepts to enhance current therapies is necessary. Proven leadership and personnel management skills are essential. Highly desirable attributes would include: Prior industry experience in the medical industry. Considered to be a plus: A background in and knowledge of end stage renal disease and dialysis therapies, including fluid balance, acid base balance, and clearance of uremic toxins. To be successful in this organization, the candidate must possess a collaborative interpersonal style as well as the leadership skills to excel in a matrix environment.

For more information about Baxter, please visit our website at **www.baxter.com**. To apply on the career page, reference **Job Code 18688BR**, or email your resume to: **connie_weeks@baxter.com** and reference this ad.

ASSOCIATE CHIEF OF STAFF FOR RESEARCH

VA North Texas Health Care System in Dallas, Texas seeks an Associate Chief of Staff-Research to administer and further develop the research and development program at the medical center. The successful candidate will maintain the institution's existing strengths in funded basic and clinical research, and grow the R&D program with a focus on expanding translational, patient centered and HSR&D research at the institution. The successful candidate will also continue to foster and grow the research collaboration between the VA North Texas Health Care System and the medical school affiliate, the University of Texas Southwestern Medical Center.

The successful candidate will have a history of a sustained, independent research program, with an active program preferred. A history of success in clinical, translational or HSR&D research is preferred. The candidate must have a commitment to academic medicine and education. For clinician/scientists, clinical responsibilities and service assignments are negotiable. Candidates must qualify for an academic appointment at University of Texas Southwestern Medical Center at a minimum of the Associate Professor level.

The Dallas VA Medical Center is one of the largest in the VA system with over 900,000 outpatient visits and 13,000 hospital admissions in 2004. The Dallas/ Fort Worth Metroplex is a cosmopolitan, vibrant metropolitan area with excellent cultural opportunities, professional sports and world class dining and shopping. A low cost of living, no state income tax and superb national and international access courtesy of DFW airport are major strengths of the metroplex.

Review of applications will begin immediately and will continue until the position is filled or the search closed. Candidates should forward their Curriculum Vitae and three references to: **George A. Sarosi, M.D., Chair, Search Committee, c/o Surgical Service (112), VA North Texas Health Care System, 4500 S. Lancaster Road, Dallas, Texas 75216; Email: georgeajr.sarosi2@med.va.gov.**

VA NORTH TEXAS HEALTH CARE SYSTEM
Equal Opportunity Employer

National Oceanic and Atmospheric Administration (NOAA)

Director, Air Resources Laboratory (ARL)

(A Senior Executive Service Position in the Federal Government)
Vacancy Announcement NOAA#06-12

Department of Commerce (DOC)
National Oceanic and Atmospheric Administration (NOAA)
Office of Oceanic and Atmospheric Research (OAR)
Silver Spring, Maryland
$109,808 - $152,000 annually

The candidate selected for this position is responsible for providing expert scientific and technical direction in the areas of research on air quality, atmospheric dispersion, and climate, with an emphasis on considering the air as a part of the total environment. ***The applicant must possess the following:***

1. Broad understanding of the scientific foundations and research challenges relevant to the Air Resources Laboratory (ARL) and NOAA mission. The incumbent should have a solid grounding in the physical sciences and supporting technical and scientific tools.

2. Demonstrated competence in atmospheric sciences sufficient to perceive complex problems in a broad perspective, to infuse ideas, and to participate meaningfully in direction and evaluation of atmospheric research.

3. Demonstrated ability to lead senior-level research in areas related to the ARL and NOAA mission, and relationships with terrestrial and aquatic environmental sciences.

Please contact **Norma Hughes at 301/713-6307** for an announcement package **(Internet: address: norma.j.hughes@noaa.gov),** including mailing instructions—referring to the announcement number -OR- you may access the entire full-text vacancy from NOAA's Executive Resources Homepage (see below).

Incomplete applications will be returned.
http://www.hr.noaa.gov/er-home.htm
This vacancy will close on May 24, 2006
"NOAA Values a Diverse Workforce and is an Equal Opportunity Employer"

CENTRAL DRUG RESEARCH INSTITUTE
(Council of Scientific & Industrial Research)
Chattar Manzil Palace, P.O. Box 173, Lucknow-226 001 (India)

ADVERTISEMENT NO. 1/2006

Applications on the prescribed forms are invited from the persons of Indian nationality for the following posts in Central Drug Research Institute, Lucknow.

1. Scientist Gr. IV(5) : One Post (for Pharmaceutics Division): Scale of Pay : Rs. 16,400-450-20,000/-): Essential Qualification: Ist Class M.Sc. or equivalent in any branch of science including Pharmacy with Ph.D. in Pharmaceutical Sciences and atleast 10 years Post-doctoral research experience in the area of new drug formulations and delivery systems as evidenced by outstanding record of publications in high impact journals. Candidates possessing atleast 5 years of independent R&D experience in the above areas and quality control of drugs and pharmaceuticals shall be given preference. This is a very senior position where the candidate is expected to provide leadership to R&D group devoted new formulations and delivery systems, and also to participate in institutional management.

2. Scientist Gr. IV(3) Four posts:
Scale of Pay: Rs.12000-375-16500/-
Post No. 1 (for Pharmacology/Endocrinology Division);
Post No. 2 (for Pharmacology/Endocrinology Division);
Post No. 3 (for Parasitology Division);
Post No. 4 (for Endocrinology Division):

3. Scientist Gr. IV(2) : Eight posts:
Scale of Pay : Rs. 10000-325-15200/-):
Post No. 1 (for Pharmacokinetics Division); Post No. 2 (for Pharmaceutics Division) ; Post No. 3 (for Pharmaceutics Division); Post No. 4 (for Pharmacology/Endocrinology Division); Post No. 5 (for Pharmacology/ Endocrinology Division); Post No. 6 (for Medicinal & Process Chemistry Division); Post No. 7 (for Medicinal & Process Chemistry Division); Post No. 8 (for Endocrinology Division)

Candidates already applied earlier against these posts need not apply again.

For detailed information Website : http://www.cdriindia.org/situationv.asp may be referred to.

Associate Dean, Research
College of Agricultural, Consumer and Environmental Sciences (ACES)
University of Illinois at Urbana-Champaign

Position Description: The Associate Dean, Research provides leadership and administrative oversight of the domestic and international research programs of the College of ACES and is responsible for management of the research activities of the Illinois Agricultural Experiment Station. The Associate Dean, Research reports to the Dean of the College and works closely with the Associate Dean, Academic Programs, and the Associate Dean, Extension and Outreach, as well as the department heads within the College, to encourage and support the development and maintenance of a strong, dynamic research organization.

Responsibilities: Provide visionary leadership for research and scholarly activities; Facilitate continued faculty excellence in research including team building of multi-disciplinary, multi-functional, multi-institutional and/or international programs; Develop new research capacity and associated infrastructure; Champion ACES research in scientific communities on and off campus; Stimulate formation of innovative research ideas and translation of research into practice; Play a key role in College-level decisions concerning resource allocation and advise the Dean on promotion and tenure; Utilize and invest Office of Research resources to advance scholarship.

Qualifications: Applicants must have a doctorate in a relevant field of study and qualify for a tenured faculty appointment at the rank of full professor in a College department. Desired qualifications include a recognized national and international scholarly reputation, leadership and/or administrative experience, strong interpersonal and organizational skills, an understanding of the broad mission of a land grant university, a demonstrated commitment to international activities, and acknowledgement of the importance of diverse disciplines represented by ACES research programs.

Salary: Competitive and commensurate with experience and qualifications.

Proposed Starting Date: Negotiable – approximately January, 2007

Applications: To ensure full consideration, candidates should apply by **June 15, 2006**. See **http://www.aces.uiuc.edu/Announcements/index.cfm** for full job description and application instructions.

Contact: Dr. K. C. Ting, Chair, Phone: 217-333-3570; Fax: 217-244-0323; E-mail: kcting@uiuc.edu.

The UIUC is an AA/EOE.

POSITIONS OPEN

FACULTY POSITION
Center for Pharmacogenetics
Department of Pharmaceutical Sciences
School of Pharmacy
University of Pittsburgh

We are seeking to fill a tenure-track faculty position at the rank of **ASSISTANT PROFESSOR** in any area of biomedical research, although candidates working in pharmacogenetics/pharmacogenomics are particularly encouraged to apply. The successful candidate should have a Ph.D., postdoctoral training, and clear potential for NIH funding. The Center for Pharmacogenetics in the Department of Pharmaceutical Sciences is well equipped to perform state-of-the-art basic and translational research. The University of Pittsburgh and the School of Pharmacy have been consistently ranked nationally among the top 10 in NIH funding. Existing research programs include nuclear receptor-mediated gene regulation in drug development, liver disorders, and metabolic diseases; gene therapy and pharmacotherapy of cardiovascular and pulmonary disorders; molecular biology of protein degradation and its physiological and disease relevance; mouse genetics; and proteomics. For more information about the Center and Department, visit our **website: http://www.pharmacy.pitt.edu/research/pharmacogen/.**

Applicants should submit a letter of interest, complete curriculum vitae, a two-to-three page description of future research plans, and at least three letters of reference to: **Dr. Wen Xie, Interim Director, Center for Pharmacogenetics, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261; e-mail: wex6@pitt.edu.**

The University of Pittsburgh is an Affirmative Action, Equal Opportunity Employer.

POSTDOCTORAL POSITIONS
Cancer Biology Training Program
Indiana University School of Medicine

The Cancer Biology Training Program, supported by fellowships from the NIH, seeks qualified postdoctoral students who wish to pursue a career in basic or translational cancer research. In addition to their research experience, trainees are exposed to a broad range of cancer-related topics encompassing both basic and clinical aspects of the disease. Visit **website: http://www.iupui.edu/~micro/body_cancer_biology.html** for a list of research areas and mentors and more information on the Program. *Candidates must be U.S. citizens or permanent residents.* Applicants are invited to contact faculty listed on the program website, and should also send curriculum vitae with three references to: **Ann Roman, Ph.D., Microbiology and Immunology, Indiana University School of Medicine, 420 MS, 635 Barnhill Drive, Indianapolis, IN 46202-5120, e-mail: aroman@iupui.edu.** *Indiana University is an Affirmative Action/Equal Opportunity Employer. Qualified minority applicants are encouraged to apply.*

POSTDOCTORAL SCIENTISTS AND RESEARCH ASSOCIATE SCIENTISTS
Molecular Determinants of Cancer

Positions available to characterize novel genes mediating tumorigenesis, cancer progression and suppression, apoptosis, differentiation, and senescence. Training in molecular biology and biochemistry required. Experience with transgenic mice, protein analysis, and purification or protein-protein interactions desirable. Send curriculum vitae with reference list to: **Dr. Paul B. Fisher, Columbia University, College of Physicians and Surgeons, BB 15-1501, 630 West 168th Street, New York, NY 10032. E-mail: pbf1@columbia.edu.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

University of Michigan
Kellogg Eye Center

ASSOCIATE PROFESSOR OR PROFESSOR
University of Michigan
Retina Translational Research

In anticipation of expanded programs, the Department of Ophthalmology and Visual Sciences at the University of Michigan is seeking candidates for a new Clinician Scientist position in the field of age-related macular degeneration and/or retinal degeneration. This person will help lead efforts in translational research for effective treatments for retinal degeneration and will join a very strong clinical and basic science retinal research group. Candidates are expected to have an established track record of research funding and clinical trials. Letters of interest with summary of research plans and curriculum vitae can be sent to: **John R. Heckenlively, M.D., Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105.** *The University of Michigan is an Equal Opportunity Employer. We are building for the future. A nondiscriminatory, Affirmative Action Employer.*

COMPUTATIONAL SEISMOLOGY/IMAGING

The Department of Geophysics at Stanford University seeks applicants for a tenure-track position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in seismology or geophysical imaging at the regional to global scale. Priority will be given to candidates who use and develop advanced computational methods, will make novel use of newly available data sets, and will interact closely with existing programs in exploration seismology, geophysical imaging and the Center for Computational Earth and Environmental Science. We are looking for an individual with a commitment to excellence in both research and teaching. Applications, including curriculum vitae, a statement outlining research and teaching experience and interests, and the names and addresses of three references should be sent in either electronic (PDF only) format or paper. Initial review of applications will begin on April 30, 2006, but the position will remain open until filled.

Please send electronic applications to **e-mail: geophysics-search@pangea.stanford.edu,** or if preferred, paper applications to:

Chair, Geophysics Search Committee
Department of Geophysics
Mitchell Building, 360
Stanford University
Stanford, CA 94305-2215 U.S.A.

Additional information about School of Earth Sciences, Stanford University, can be found on its **website: http://pangea.stanford.edu/.**

Stanford University has a strong institutional commitment to the principle of diversity. In that spirit, we particularly encourage applications from women, members of ethnic minorities, and individuals with disabilities.

TWO CONSULTANT POSITIONS
The University of Minnesota
Supercomputing Institute

PROTEOMICS/PROTEIN SCIENCE CONSULTANT. Have a background in computational biology, chemistry, or molecular biophysics? Consult with University of Minnesota researchers to aid in effective resolution of proteomics-related research problems. Provide expertise with strategic planning and evaluation of hardware and software technology.

BIOINFORMATICS CONSULTANT. Have experience in bioinformatics, databases, development? The University of Minnesota Supercomputing Institute is looking for a Consultant to work with University researchers to create effective support for bioinformatics, including creating strategic plans, evaluating hardware and software, and presenting tutorials.

For details on these jobs and application information, please see the employment section at **website: http://www.msi.umn.edu** or contact **Ann Johns** at **e-mail: johns@dtc.umn.edu.** *The University of Minnesota is an Equal Opportunity Educator and Employer.*

Tulane University
School of Public Health and Tropical Medicine

Louisiana Cancer Research Consortium
OF NEW ORLEANS

Environmental Health Sciences
Tenure Track Faculty Positions/Program Leadership

The Louisiana Cancer Research Consortium and the Department of Environmental Health Sciences - Tulane School of Public Health and Tropical Medicine are seeking applications for two tenure-track faculty positions at the Associate Professor level. Candidates with emphasis in Mutagenesis (environmentally induced carcinogenesis), and Environmental Epidemiology of Cancer will be considered. The Department and Cancer Center are both undergoing major periods of expansion and outstanding startup, facilities and environment will be provided. Candidates should hold a doctoral degree in a relevant discipline, post-doctoral experience, have demonstrated potential to establish independent research programs, preferably with a laboratory component, and show potential for excellence in teaching. Applicants whose research focuses on human exposures and biomarkers of cancer risk, mechanisms of carcinogenesis, or chemoprevention of cancers are of particular interest. Applicants who utilize contemporary model systems and molecular, genomic, proteomic, and metabolic approaches in their research are desirable. The successful candidates require a history of extramural support. Opportunities for Program Leadership exist.

Applicants should send a cover letter, curriculum vitae, description of research plans and three letters of recommendation to: **Dr. Maureen Lichtveld, Tulane University School of Public Health and Tropical Medicine, Department of Environmental Health Sciences - SL29, 1430 Tulane Avenue, New Orleans, Louisiana 70112**. Applications will be accepted until the positions are filled.

Tulane University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

UCSD

Lecturer with Potential for Security of Employment
University of California, San Diego
Division of Biological Sciences
http://biology.ucsd.edu/

The Division of Biological Sciences at the University of California, San Diego seeks outstanding applicants for an opening as Lecturer with Potential for Security of Employment in Biochemistry. All interested parties are encouraged to apply, including minorities and women.

The Lecturer will be responsible for teaching and coordinating the Biochemical Techniques Laboratory, the introductory lab course for Biology majors at UCSD. Multiple sections of the course are offered each quarter, and it will be the responsibility of the Lecturer to develop and provide the curriculum for other instructors teaching the course. In addition the successful candidate will be responsible for training of new laboratory Teaching Assistants and provide service to the University by participating in committees and recruitment and outreach efforts.

A Ph.D. in a relevant field of science is required. Applicants must have a strong record of excellence and innovation in teaching, broad-based experience in biochemistry, along with strong interpersonal, writing and computer skills. The position will be a nine-month appointment. Salary will be commensurate with background and experience and based on University of California pay scale. A Lecturer-PSOE position closely parallels that of an assistant professor on track for tenure.

Complete applications received by **May 31, 2006** will be assured consideration. Applicants should send a curriculum vitae, publication list, synopsis of professional goals, and three letters of reference (forwarded separately) to:

Lecturer Search Committee
c/o April Hunter – Mail Code 0355-B
University of California, San Diego
9500 Gilman Drive
La Jolla, CA 92093-0355

UCSD is an Equal Opportunity-Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.

HARVARD MEDICAL SCHOOL
MASSACHUSETTS GENERAL HOSPITAL
MassGeneral Institute for Neurodegenerative Disease

RESEARCH SCIENTIST
Massachusetts General Hospital
Department of Neurology

The Cecil B. Day Laboratory for Neuromuscular Research at Massachusetts General Hospital is seeking a Masters level or Ph.D. scientist to participate in studies of non-viral technologies to improve the delivery of proteins to the central nervous system (CNS). The candidate will work with a growing team of investigators to accelerate protein therapy for brain and spinal cord disorders, with a particular focus on degenerative disorders such as amyotrophic lateral sclerosis.

Applicants must have demonstrated productivity in stereotactic surgical techniques required for implantation of intracerebroventricular and intrathecal cannulae in mice. Expertise in processing of CNS tissues for immunohistochemical and ELISA analyses is also highly desired. Salary is competitive and negotiable depending on previous experience. Candidates with Ph.D. degrees will be research fellows both at the Massachusetts General Hospital and at Harvard Medical School. Our laboratory is part of the newly established Massachusetts General Hospital Institute for Neurodegenerative Disease located just outside of Boston in the Charlestown Navy Yard. Several large universities and many biotech companies make Boston an exciting biomedical community with diverse opportunities for scientific collaborations. In addition, Boston is near excellent beaches and ski areas.

Interested applicants should send curriculum vitae, names of three references, and a cover letter indicating research interests and experience to: **Jonathan W. Francis, Day Neuromuscular Research Laboratory, Massachusetts General Hospital, Building 114, 16th Street, Room 3125, Charlestown, MA 02129**.

The Massachusetts General Hospital is an Equal Opportunity Employer.

POSITIONS OPEN

POSTDOCTORAL FELLOWSHIPS
Signal Transduction and Cancer

The Robert H. Lurie Comprehensive Cancer Center of Northwestern University is seeking candidates for Postdoctoral positions in an NCI funded program, Signal Transduction and Cancer. In addition to laboratory research, trainees participate in journal clubs, seminars, and symposia to prepare them for independent careers in the field. Examples of participating faculty include: **Vincent Cryns, Margarita Dubocovich, Kathleen Green, Chung Lee, Jill Pelling, Steven Rosen, M. Sharon Stack, Teresa Woodruff.** Candidates *must be U.S. citizens or permanent residents*, interested in a career in cancer research, and must have received a Ph.D. in the biological sciences in the last two years. Applicants should send curriculum vitae, letter of reference, graduate transcript, and a statement of research interests to: **Robin Leikin, Ph.D., Administrative Director, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, 303 E. Chicago Avenue, Chicago, IL 60611. E-mail: rleikin@northwestern.edu.** For additional information, see **website: http://www.cancer.northwestern.edu/Education/Training.cfm.** *Northwestern University is an Equal Opportunity/Affirmative Action Employer. Minorities are strongly encouraged to apply.*

POSTDOCTORAL POSITION
Quantitative Toxicology/Pharmacology

The U.S. Environmental Protection Agency (EPA), National Center for Environmental Assessment in Cincinnati, Ohio, seeks Postdoctoral candidates in computational toxicology and biological modeling for human health risk assessments. Successful candidates will have experience in some of the following areas: toxicology, biochemistry, physiology, pharmacology, statistics, and computer modeling. Specialized education training and/or experience preferred include quantitative structure-activity (e.g., QSAR, SAR) modeling, pharmaco- or toxicokinetic modeling, or physiological or biologically-based dose response modeling. Salary ranges from $50,000 up to $70,000, commensurate with qualification plus benefits. Information on federal positions can be found at **website: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=134123 (CINCSAST-010406-01).** For additional information contact **Dorothy Carr** at **telephone: 919-541-4356.** Information on nonfederal positions can be found at **website: http://www.orau.gov/orise/edu/needs/EPA-NCEA-2006-01.pdf.** For additional information please contact **Karen Proffitt** at **telephone: 513-569-7099.**

US EPA is an Equal Opportunity Employer.

POSTDOCTORAL TRAINING
Vascular Cell Biology

Positions are available on a NIH funded training grant (*restricted to U.S. citizens or permanent residents*) in the laboratories of the following faculty: **Drs. Edward Plow, Paul DiCorleto, Guy Chisolm, Diane Perez, Jane Hoover-Plow, Donna Driscoll** and **Joan Fox.** Please see faculty web pages **(websites: http://www.lerner.ccf.org/moleccard/faculty/** or **http://www.lerner.ccf.org/cellbio/faculty/)** for description of research interests in each laboratory. We encourage women and minority candidates to apply. Applicants should send their curriculum vitae and the names and contact information for three references to: **the Program Director, Dr. Edward Plow, e-mail: plowe@ccf.org.**

POSTDOCTORAL RESEARCH FELLOW sought to study the genetic basis of microcephaly. Requirements include M.D. with significant experience in pediatric neurology including ability to perform clinical analysis of research subjects, gene mapping, and cloning. Salary $70,000 per year. Send resumes to: **Christopher Walsh, M.D., Ph.D., Department of Neurology, Howard Hughes Medical Institute and Beth Israel Deaconess Medical Center, NRB 266, 77 Avenue Louis Pasteur, Boston, MA 02115 (e-mail: walshlab@hotmail.com).**

POSITIONS OPEN

Nestlé

POSTDOCTORAL FELLOWSHIP
Plant Molecular Biology/Cell Biology

The Nestle Research and Development Centre in Tours, France invites applications for a Postdoctoral Fellowship in plant molecular biology. The candidate will join a team studying genes associated with coffee grain development and coffee quality (see **Lin et al.,** *Theor. Appl. Genet.* **112:** 114, 2005). Applicants should possess a recent Ph.D. and have broad experience in plant molecular biology as evidenced by published research in the field. Experience in other areas such as plant cell culture, seed biochemistry, and bioinformatics would be a plus. The successful candidate will be a creative, self-motivated individual with the ability to work and communicate efficiently with both research colleagues and business managers. The position will initially be for 18 months. For consideration, please send a resume with the names of three references, and a cover letter expressing your interest by post to: **Manuela Guerin, Nestlé Research and Development Centre, 101 Avenue Gustave Eiffel, 37097, BP 49716, Tours, France.**

The Flow Visualization Laboratory at the McGowan Institute for Regenerative Medicine, University of Pittsburgh, invites applications from qualified individuals for a **POSTDOCTORAL POSITION.** Highly qualified candidates may be considered for an appointment at research **ASSISTANT PROFESSOR.** Applicants are expected to have experience in flow visualization and digital particle imaging velocimetry. The successful candidate must have strong communication skills and background in fluid mechanics. Knowledge in biomedical devices is desired, but not required. The candidate will have opportunities to interact with colleagues in the Department of Bioengineering **(website: http://www.engr.pitt.edu/bioengineering)** and the McGowan Institute **(website: http://www.mirm.pitt.edu)** on research projects related to cardiovascular flow and devices. The position requires an earned doctoral degree in engineering or a closely related engineering discipline, or a doctoral degree in a related physical science. Applicants should submit their vitae and a list of three references to: **Nicole Johnson, McGowan Institute, 3025 E. Carson Street, Pittsburgh, PA 15203.** Electronic submission strongly encouraged. Materials should be sent to **e-mail: johnsonnm2@upmc.edu.** Questions and inquiry can be sent to **e-mails: joul@upmc.edu** or **federspielwj@upmc.edu.**

POSTDOCTORAL FELLOW

Postdoctoral positions are available to study the molecular regulation of corneal epithelial wound healing in the Kresge Eye Institute, Wayne State University School of Medicine, Detroit, Michigan. Candidates should be highly motivated with a solid background in molecular and cell biology. Please send curriculum vitae with a list of references to **Dr. Fu-Shin Yu, e-mail: fyu@med.wayne.edu.** *Wayne State University and the Kresge Eye Institute are Equal Opportunity Employers.*

EUKARYOTIC CELL BIOLOGIST. Winona State University (Minnesota) seeks a Biologist to teach undergraduate courses in cell biology, genetics and principles of biology. Ph.D. or equivalent in bilogy or related field. For additional information, see **websites: http://www.winona.edu/humanresources,** Employment Opportunities, and **bio.winona.edu/thompson/cellsearch.htm.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

POSTDOCTORAL FELLOW POSITIONS
Institute of Molecular Medicine and Genetics
Medical College of Georgia

Postdoctoral positions with possible promotion to a faculty position are available immediately. Our laboratory is focusing on the mechanisms that regulate the expression of human beta-like globin genes (*PNAS* **98:**1847, 2001, *Exp. Hem.* **32:** 244, 2004). First position is to study intracellular signaling pathways that regulate expression of the fetal hemoglobin gene in a stage-specific manner. Experimental approaches include reporter gene assays, protein expression, and analysis of protein-DNA and protein-protein interactions such as footprinting, chromatin immunoprecipitation, and chromosome con-formation capture assays. The second position will study the mechanism for fetal hemoglobin gene expression by creating transgenic mice. These positions require a strong background of molecular biology techniques as well as extensive knowledge on intracellular signaling or experience of handling mice and controlling mouse colonies. Interested candidates please: Apply online at **website: http://www.mcg.edu/Jobs/Apply/.** Job DJP001779-00007489. For more information, please contact: **Dr. Tohru Ikuta** at **e-mail: tikuta@mcg.edu.** *Affirmative Action/Equal Employment Opportunity/Equal Access/ADA Employer.* E-0684070.

RESEARCH PATHOLOGIST

Full-time position available at Fox Chase Cancer Center, Philadelphia, Pennsylvania, to manage Laser Capture Microdissection (LCM) core facility. Successful candidate will perform LCM, train investigators in the use of two LCM workstations, and provide histopathological expertise. Three or more years of surgical pathology training and experience in molecular biology required. Please send curriculum vitae with names and contact information of two references to **e-mail: kathy.ireton@fccc.edu.** *Equal Opportunity Employer.*

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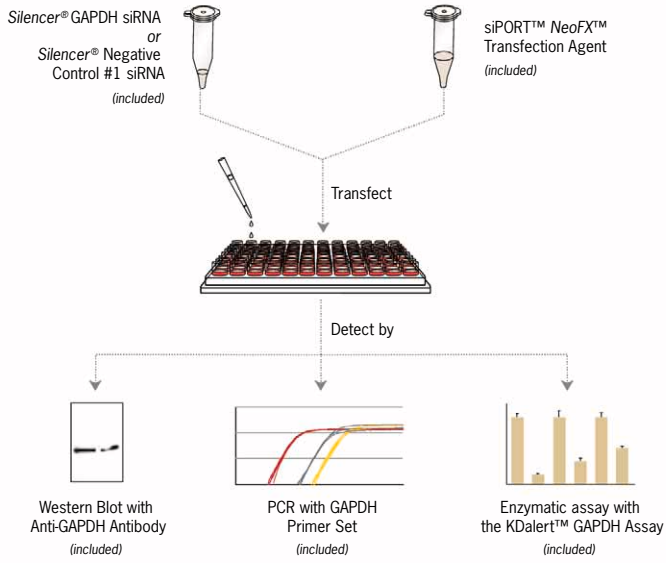

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